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19. ABSTRACT (Continue on reverse if necessary and identify by block number)  A total of 3,514 punch-necropsy-specimens (voles) have been tested. The results on both the Puumala virus antibody and antigen ELISA tests, as well as information on the specific trapping date, location, species, and weight, have been entered into a computer database. Monoclonal antibodies to Puumala virus are currently being developed. Mouse hybridomas have been fused with B-cells from Clethrionomus glareolus. Approximately 100 hybridomas have made antibodies to Puumala virus; a few have been recloned and appear to be stable.					
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## FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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## INTRODUCTION

Nephropathia epidemica (NE), a member of the hemorrhagic fever with renal syndrome (HFRS), causes considerable human morbidity in Scandinavia and western USSR. The etiological agent of NE, Puumala virus (PUU), was isolated in 1983 from a bank vole (Clethrionomys glareolus). Recently a nation-wide study of NE was conducted in Sweden, identifying major endemic areas of the disease. This study indicated that C. glareolus is the most important vector of PUU virus in Sweden. The basic biology of this species is thus directly related to the probability of disease in humans. C. glareolus is the most abundant small mammal in Sweden and is commonly found from the southern tip of the country northward to near the arctic circle. Although only one subspecies of C. glareolus is recognized throughout the Swedish mainland, a gradient exists in population stability.

Populations of C. glareolus in the south of Sweden are non-cyclic, whereas those in the north fluctuate on a three- to four-year cycle of abundance. During peak populations, voles may be 1.000 times more abundant than immediately after population crashes. The number of human cases in the endemic area correlates strongly to the fluctuation of C. glareolus.

There is a boundary between the cyclic population in the north and the more or less non-cyclic population in the south. This boundary corresponds to the limes norrlandicus, a bio-geographical line running from 59°N on the west coast of Sweden to 61°N on the eastern shore. This line separates the northern boreal (or taiga) zone from the southern boreo-nemoral zone. The limes norrlandicus also appears to demarcate the southern distribution boundary of NE in humans and of PUU virus in voles. Antibody prevalence as well as disease incidence in humans increase to the north of the limes norrlandicus, reaches a peak in a region near the 64°N parallel and then decrease in the far north. Infection-rates in C. glareolus follow the same pattern.

Since C. glareolus is the most important vector of PUU virus the ecology and biology of this vector is therefore the key for in-depth understanding of the epidemiology of the disease. The aim of the present study was to map infection rate in bank voles over time in a well defined endemic area of Sweden.

## **MATERIAL AND METHODS**

### **Animals**

Small rodents in this study were collected between 1979 and 1986 by the National Environment Protection Board at 16 different specified sites in an highly endemic area (Västerbotten (AC) county 64 N, 20 E) of Sweden. This collection has been repeated at exactly the same locations twice every year during the same weeks in May and September since 1971. At each trapping occasion 2840 traps have been placed during 3 days (8520 "trap-nights"). Animals were trapped in snap-traps, collected twice a day and stored at -20 C until processed in the laboratory.

Species, approximate age (weight), date and location of trapping were available.

Lung tissue from all animals were investigated for presence of PUU virus antibody and antigen. Two punch-necropsies of 2,0 mm (in diameter) were taken through the thorax of the animal on each side. The 4 necropsies were grinded in 2 ml of PBS using mortar and pestle.

### **Immune reagents used in antibody and antigen ELISA.**

Antigen was prepared from PUU virus (strain Vindeln 83-223L) infected Vero E-6 cells (CRL 1586; ATCC, Rockville, Md). The preparations of PUU virus antigen, negative control antigen and rabbit immune serum are described elsewhere.

Human immunoglobulin to PUU virus were prepared by ammonium precipitation.

### **Detection of PUU virus specific IgG antibodies by ELISA.**

A sandwich ELISA was employed as follows. Rabbit anti-PUU virus immunoglobulin diluted 1:400 in coating buffer (0,05 M sodium carbonate, pH 9,5 to 9,7) was absorbed to 96 well polystyrene microtiter plates (Cooke M 29 AR Dynatech laboratories) at 37 C for 1 h, followed by virus antigen (diluted 1:4 at 37 C for 1 h), grinded lung specimen (diluted 1:2 in ELISA buffer (phosphate-buffered saline without Mg and Ca and with 0,05% Tween 20 and 0,5% bovine serum albumine) at 37 C for 1 h, and goat anti-mouse IgG conjugated with alkaline phosphatase diluted 1:600 in ELISA buffer at 37 C for 1 h. P-nitrophenol-phosphate (Sigma) diluted in diethanolamine buffer (1 M diethanolamine pH 9.8, 0.5 mM MgCl<sub>2</sub>) was used as the substrate. Washing between each step was done 6 times in washing buffer (saline with 0.05% Tween 20). The reaction was read after 30 min at room temperature in a spectrophotometer at 405 nm and expressed as optical density (OD). Optimal dilutions of all reagents used in the ELISA were determined by box titrations. All specimens were tested in duplicate with antigen and negative control antigen. The OD was calculated as the average OD with antigen minus the average OD with negative control antigen. To adjust for plate-to-plate and test-to-test variations in the assay, a positive control serum was included on all plates. This control had an OD of 0.700 (in the linear interval of this IgG ELISA). If the positive control serum had an OD between 0.500 and

0.900 the plate was accepted: however, all OD values on that plate were multiplied by factor so as to set the positive control value at 0.700.

**Detection of PUU virus specific antigen by ELISA.**

The testformat for the antigen are described in detail in a previous publication. In short, human anti PUU Ig was absorbed to the wells. Grinded lung specimen (diluted 1:2 in ELISA buffer) were added, followed by peroxidase labelled human anti PUU and TMB substrate. The reaction were stopped after 5 min by  $H_2SO_4$ . The optical density was read at 450 nm at room temperature. To adjust for plate-to-plate and test-to-test variations in the assay the procedure described above for IgG ELISA was used.

## RESULTS.

A total of 3525 rodents were tested including 2493 C glareolus, 620 C rufocanus, 367 Microtus agresis, 39 Myop Sch and 5 Apodemus flavicollis. The overall proportion of antigen and antibody positive animal for the 4 most common species are shown in figure 1 and 2 respectively.

Additional analyses of these results are included in the accompanying tables and figures. Results depicted here are still under investigation and will be summarized in subsequent reports.



## DISCUSSION

Abundance of small mammal in relation to environmental variables have been investigated by several scientists in Scandinavia. A theory have been put forward saying that the fluctuation of small mammals correlates with a "green-index" probably meaning the chlorofyll-mass as well as with the size of the tree-seeds in the area. The peaks would occur when the greenindex is the highest. The investigators claims that they have seen this correlation and that it seems feasible since greenindex and seed-size would correlate with food-supply for the small mammals. The magnitude of the peaks due to this theory depends on both the lengths of the snow-cover since the snow protect small mammals from predators and on soil-temperature. The longer the snowcover and the warmer the soil-temperature the higher the peak that specific year.

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TABLE 1

# AG AND AB IN DIFFERENT VOLE SPECIES

	CLET GLA	CLET RUF	MICR AGR	MYOP SCH	APOD FLA
TOTAL NUMBER OF SPEC	2493	620	367	39	5
NUMBER OF AG NEG	2171	598	346	35	5
NUMBER OF AG POS	322	22	21	4	0
NUMBER OF POS AG(%)	12,9	3,5	6,1	10,2	0
NUMBER OF AB NEG	2015	562	349	38	5
NUMBER OF AB POS	478	58	18	1	0
NUMBER OF POS AB(%)	19,2	9,4	4,9	2,6	0
POS AG/POS AB RATIO	0,674	0,379	1,167	4,000	-

CUTOFF FOR AG: OD=70

CUTOFF FOR AB: OD=180

TABLE 2

**AVERAGE WEIGHT AND NUMBERS, PER SEASON AND YEAR  
CLET GLA**

	AVERAGE WEIGHT OF SPECIMENS (g)	NUMBER OF SPECIMENS
F79	157	40
S80	225	38
F80	172	463
S81	233	81
F81	186	500
S82	235	57
F82	146	90
S83	213	11
F83	153	178
S84	233	129
F84	178	691
S85	206	23
F85	159	44
S86	224	17
F87	180	131
ALL SPRING CAPTURES	230	356
ALL FALL CAPTURES	174	2,137

S=SPRING F=FALL

TABLE 3

The table shows the total number of specimens, the number of antigen positive specimens, and the number of antibody positive specimens per season and year. The percentage positive antigen and antibody, and the ratio between positive antigen and antibody are given.

	SPRING CAPT.	1979 FALL CAPT.	SPRING+ FALL	SPRING CAPT.	1980 FALL CAPT.	SPRING+ FALL
TOTAL NUMBER		40		38	463	501
AG NEG		38		36	443	479
AG POS		2		2	20	22
% AG POS		5.0%		5.3%	4.3%	4.4%
AB NEG		37		33	359	392
AB POS		3		5	104	109
% AB POS		7.5%		13...%	22.5%	21.8%
POS AG/AB RATIO		.667		.400	.192	.202
	1981			1982		
TOTAL NUMBER	81	500	581	57	90	147
AG NEG	71	406	477	49	74	128
AG POS	10	94	104	8	16	24
% AG POS	12.3%	18.8%	17.9%	14.0%	17.8%	16.3
AB NEG	55	364	419	36	78	114
AB POS	26	136	162	21	12	33
% AB POS	32.5	27.2%	27.9%	36.8%	13.3%	22.4
POS AG/AB RATIO	.385	.691	.642	.381	1.333	.727
	1983			1984		
TOTAL NUMBER	11	178	189	129	692	821
AG NEG	10	161	171	108	587	695
AG POS	1	17	18	21	105	126
% AG POS	9.1%	9.6%	9.5%	16.3%	15.2%	15.3%
AB NEG	9	157	166	96	608	704
AB POS	2	21	23	33	84	117
% AB POS	18.2%	11.8%	13.9%	25.6%	12.1%	14.3%
POS AG/AB RATIO	.500	.810	.782	.636	1.250	1.077

TABLE 3, CONT

	SPRING CAPT.	1985 FALL CAPT.	SPRING+ FALL	SPRING CAPT.	1986 FALL CAPT.	SPRING+ FALL
TOTAL NUMBER	23	44	67	17		
AG NEG	17	36	53	14		
AG POS	6	8	14	3		
% AG POS	26.1%	18.2%	20.9%	17.6%		
AB NEG	16	39	55	13		
AB POS	7	5	12	4		
% AB POS	30.4%	11.4%	17.9%	23.5%		
POS AG/AB RATIO	.857	1.600	1.167	.750		
	1987					
TOTAL NUMBER		130				
AG NEG		121				
AG POS		9				
% AG POS		6.9%				
AB NEG		115				
AB POS		15				
% AB POS		11.5%				
POS AG/AB RATIO		.600				

TABLE 4

The table shows the total number of specimens, the number of Ag and Ab positive specimens, and the percent positive Ag and Ab per weight class. The table also gives the mean positive OD and the ratio between positive Ag and Ab for each weight class.

WEIGHT INTERVAL (g)	TOT NUMB	AG POS	% AG POS	MEAN OD, AG	AB POS	% AB POS	MEAN OD, AB	RATIO AG/AB
1-25	3	0	0	0	1	33.3	281	-
26-50	4	0	0	0	0	0	0	-
51-75	12	0	0	0	0	0	0	-
76-100	21	1	4.8	123	2	9.5	594	.500
101-125	76	5	6.6	280	8	10.5	549	.625
126-150	349	30	8.6	371	46	13.2	469	.652
151-175	745	75	10.1	319	95	12.8	526	.789
176-200	641	88	13.7	303	109	17.0	659	.807
201-225	264	50	18.9	300	76	28.8	697	.658
226-250	201	37	18.4	340	72	35.8	666	.514
251-275	116	26	22.4	351	42	36.2	828	.619
276-300	40	9	22.5	402	22	55.0	764	.409
301-325	17	1	5.9	72	4	23.5	923	.250
326-350	2	0	0	0	0	0	0	-
351-375	1	0	0	0	0	0	0	-

TABLE 5

The table shows, for each capture season and year, the number of Ag negative, and the number of Ag positive specimens per area. The total number of Ag negative and positive specimens, as well as the percent positive Ag (of the total number of specimens) are also shown. Note: S=spring and F=fall.

AREA	F79		S80		F80		S81		F81	
	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS
21J2C	9	0	9	2	53	1	6	1	26	17
21J7C	3	0	4	0	63	2	2	1	44	2
21J2H	0	0	0	0	19	5	1	0	50	11
21J7H	2	0	0	0	9	0	10	0	37	7
22J2C	1	0	1	0	21	0	10	0	14	3
22J7C	0	0	0	0	22	4	7	0	19	6
22J2H	0	0	0	0	29	0	8	3	25	2
22J7H	11	0	4	0	83	5	8	1	52	3
21K2C	0	0	0	0	5	0	0	0	28	5
21K7C	0	0	0	0	9	0	3	3	20	10
21K2H	0	0	0	0	0	0	0	0	18	6
21K7H	0	0	0	0	6	0	2	0	9	7
22K2C	1	0	1	0	30	0	3	0	12	10
22K7C	1	0	1	0	27	0	0	0	18	2
22K2H	1	0	7	0	9	0	6	0	15	1
22K7H	9	2	9	0	58	3	5	1	18	2

AREA	S82		F82		S83		F83		S84	
	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS
21J2C	10	0	18	1	3	0	37	2	19	5
21J7C	1	1	4	2	3	0	28	3	13	4
21J2H	4	0	1	0	0	0	1	0	1	1
21J7H	5	0	2	0	0	0	0	0	0	0
22J2C	0	0	3	0	0	0	3	0	5	1
22J7C	5	0	3	1	0	0	7	1	0	0
22J2H	4	1	3	0	0	0	1	2	4	0
22J7H	12	3	19	9	2	0	36	5	17	6
21K2C	2	0	5	2	0	0	2	0	3	3
21K7C	3	2	2	0	2	0	3	0	7	0
21K2H	0	0	1	0	0	0	2	0	4	0
21K7H	1	0	2	1	0	1	3	0	11	0
22K2C	0	0	3	0	0	0	7	4	2	0
22K7C	0	0	1	0	0	0	3	0	1	0
22K2H	0	0	5	0	0	0	7	0	4	1
22K7H	2	1	2	0	0	0	21	0	17	0



TABLE 5. CONT

AREA	F84		S85		F85		S86		F87	
	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS
21J2C	69	15	1	1	5	1	2	2	0	0
21J7C	46	32	1	0	7	0	2	0	0	0
21J2H	22	9	0	0	0	0	0	0	0	0
21J7H	28	0	2	0	0	0	0	0	0	0
22J2C	16	1	1	0	2	0	0	0	0	0
22J7C	18	5	2	0	0	1	0	0	0	0
22J2H	32	1	5	0	3	0	5	0	0	0
22J7H	60	6	3	1	10	5	0	1	0	0
21K2C	42	1	0	0	0	0	0	0	9	0
21K7C	40	1	0	0	1	0	0	0	13	2
21K2H	42	8	0	1	0	0	0	0	23	0
21K7H	50	8	0	0	0	0	1	0	11	1
22K2C	28	13	1	0	0	0	0	0	16	0
22K7C	27	1	0	1	2	0	0	0	10	1
22K2H	21	3	0	1	1	1	3	0	9	0
22K7H	44	1	1	1	5	0	1	0	25	5

AREA	TOT NEG	TOT POS	%POS TOT
21J2C	267	48	15.2
21J7C	221	47	17.5
21J2H	99	26	20.8
21J7H	95	7	6.9
22J2C	77	5	6.1
22J7C	83	18	17.8
22J2H	119	9	7.0
22J7H	317	45	12.4
21K2C	96	11	10.3
21K7C	103	18	14.9
21K2H	90	15	14.3
21K7H	96	18	15.8
22K2C	104	27	20.6
22K7C	91	5	5.2
22K2H	88	7	7.4
22K7H	217	16	6.9

TABLE 6

The table shows, for each capture season and year, the number of Ab negative and the number of Ab positive specimens per area. The total number of Ab negative and positive specimens, as well as the percent positive Ab (of the total number of specimens) are also shown. Note: S=spring and F=fall.

AREA	F79		S80		F80		S81		F81	
	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS
21J2C	9	0	9	2	47	7	7	0	20	23
21J7C	3	0	3	1	44	21	1	2	40	6
21J2H	0	0	0	0	16	8	1	0	44	17
21J7H	2	0	0	0	7	2	9	1	39	5
22J2C	1	0	1	0	19	2	9	1	13	4
22J7C	0	0	0	0	21	5	5	2	13	12
22J2H	0	0	0	0	27	2	4	7	20	7
22J7H	11	0	4	0	65	23	6	3	45	10
21K2C	0	0	0	0	5	0	0	0	28	5
21K7C	0	0	0	0	6	3	2	4	14	16
21K2H	0	0	0	0	0	0	0	0	24	0
21K7H	0	0	0	0	6	0	2	0	11	5
22K2C	1	0	1	0	15	15	3	0	10	12
22K7C	1	0	1	0	23	4	0	0	16	4
22K2H	1	0	7	0	5	4	4	2	10	6
22K7H	8	3	7	2	53	8	2	4	16	4

AREA	S82		F82		S83		F83		S84	
	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS
21J2C	5	5	17	2	2	1	29	10	19	5
21J7C	1	1	3	3	3	0	30	1	12	5
21J2H	4	0	1	0	0	0	1	0	1	1
21J7H	4	1	2	0	0	0	0	0	0	0
22J2C	0	0	3	0	0	0	3	0	5	1
22J7C	4	1	4	0	0	0	8	0	0	0
22J2H	2	3	2	1	0	0	2	1	4	0
22J7H	11	4	26	2	2	0	33	8	14	9
21K2C	0	2	4	3	0	0	2	0	1	5
21K7C	1	4	1	0	2	0	3	0	6	1
21K2H	0	0	1	0	0	0	2	0	2	2
21K7H	1	0	2	1	0	1	3	0	9	2
22K2C	0	0	3	0	0	0	10	1	2	0
22K7C	0	0	1	0	0	0	3	0	1	0
22K2H	0	0	5	0	0	0	7	0	3	2
22K7H	3	0	2	0	0	0	21	0	17	0

TABLE 6

AREA	F84		S85		F85		S86		F87	
	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS
21J2C	75	9	1	1	3	3	2	2	0	0
21J7C	62	16	1	0	6	1	2	0	0	0
21J2H	23	8	0	0	0	0	0	0	0	0
21J7H	28	0	2	0	0	0	0	0	0	0
22J2C	14	3	1	0	2	0	0	0	0	0
22J7C	19	4	2	0	1	0	0	0	0	0
22J2H	25	8	4	1	3	0	4	1	0	0
22J7H	59	7	2	2	14	1	0	1	0	0
21K2C	40	3	0	0	0	0	0	0	9	0
21K7C	40	1	0	0	1	0	0	0	10	5
21K2H	43	7	0	1	0	0	0	0	22	1
21K7H	56	2	0	0	0	0	1	0	10	2
22K2C	33	8	1	0	0	0	0	0	15	1
22K7C	25	3	1	0	2	0	0	0	10	1
22K2H	20	4	0	1	2	0	3	0	9	0
22K7H	44	1	1	1	5	0	1	0	25	5

AREA	TOT NEG	TOT POS	%POS TOT
21J2C	245	70	22.2
21J7C	211	57	21.3
21J2H	91	34	27.2
21J7H	93	9	8.8
22J2C	71	11	13.4
22J7C	77	24	23.8
22J2H	97	31	24.2
22J7H	292	70	19.3
21K2C	89	18	16.8
21K7C	87	34	28.1
21K2H	94	11	10.5
21K7H	101	13	11.4
22K2C	94	37	28.2
22K7C	84	12	12.5
22K2H	76	19	20.0
22K7H	205	28	12.0

TABLE 7

The table shows the number of negative specimens, and the number of positive specimena for both Ag and Ab, per area and coordinate. The percentage positive Ag and Ab are also given.

AREA	COORD	NEG	AG POS	%POS	NEG	AB POS	%POS
21J2C	1212	78	15	16.1	71	22	23.7
	1237	8	2	20.0	10	0	0
	3712	73	16	18.0	68	21	23.6
	3737	108	15	12.2	96	27	22.0
21J7C	1212	65	14	17.7	63	16	20.3
	1237	48	6	11.1	41	13	24.1
	3712	50	10	16.7	47	13	21.7
	3737	50	17	25.4	60	15	20.0
21J2H	1212	13	2	13.3	12	3	20.0
	1237	0	0	-	0	0	-
	3712	62	21	25.3	58	25	30.1
	3737	24	3	11.1	21	6	22.2
21J7H	1212	29	2	6.5	29	2	6.5
	1237	20	3	13.0	20	3	13.0
	3712	40	2	4.8	38	4	9.5
	3737	6	0	0	6	0	0
22J2C	1212	6	0	0	6	0	0
	1237	10	1	9.1	7	4	36.4
	3712	33	1	2.9	32	2	5.9
	3737	28	3	9.7	26	5	16.1
22J7C	1212	59	10	14.5	54	15	21.7
	1237	7	1	12.5	6	2	25.0
	3712	17	7	29.2	17	7	29.2
	3737	0	0	-	0	0	-
22J2H	1212	0	0	-	0	0	-
	1237	10	2	16.7	9	3	25.0
	3712	66	7	9.6	48	25	34.2
	3737	43	0	0	40	3	7.0
22J7H	1212	87	6	6.5	77	16	17.2
	1237	79	12	13.2	78	13	14.3
	3712	81	23	22.1	80	24	23.1
	3737	70	4	5.4	57	17	23.0
21K2C	1212	7	2	22.2	6	3	33.3
	1237	29	1	3.3	28	2	6.7
	3712	52	7	11.9	47	12	20.3
	3737	8	1	11.1	8	1	11.1
21K7C	1212	29	5	14.7	24	10	29.4
	1237	19	1	5.0	19	1	5.0
	3712	13	1	7.1	9	5	35.7
	3737	42	11	20.8	35	18	34.0

TABLE 7. CONT

AREA	COORD	NEG	AG POS	%POS	NEG	AB POS	%POS
21K2H	1212	0	0	-	0	0	-
	1237	36	5	12.2	40	1	2.4
	3712	25	7	21.9	25	7	21.9
	3737	29	3	9.4	29	3	9.4
21K7H	1212	3	0	0	3	0	0
	1237	30	9	23.1	31	8	20.5
	3712	17	4	19.0	19	2	9.5
	3737	46	5	9.8	48	3	5.9
22K2C	1212	9	1	10.0	8	2	20.0
	1237	26	7	21.2	21	12	36.4
	3712	4	0	0	3	1	25.0
	3737	65	19	22.6	62	22	26.2
22K7C	1212	9	0	0	9	0	0
	1237	65	4	5.8	60	9	13.0
	3712	8	1	11.1	9	2	18.2
	3737	7	0	0	6	1	14.3
22K2H	1212	63	7	10.0	58	12	17.1
	1237	0	0	-	0	0	-
	3712	0	0	-	0	0	-
	3737	25	0	0	18	7	28.0
22K7H	1212	66	4	5.7	63	7	10.0
	1237	82	10	10.9	76	16	17.4
	3712	0	0	-	0	0	-
	3737	69	2	2.8	66	5	7.0

TABLE 8

The table shows the total number of specimens, the number of Ag positive specimens, the number of Ag negative specimens, and the percentage positive Ag per area, for two vole propagation cycles. The first cycle includes the capture seasons fall 79 - fall 82. The second cycle includes the seasons spring 83 - spring 86. The results from capture season fall 87 were not used.

AREA	CYCLE 1				CYCLE 2			
	TOT	NEG	POS	%POS	TOT	NEG	POS	%POS
21J2C	153	131	22	14.4	163	136	27	16.6
21J7C	129	121	8	6.2	139	100	39	28.1
21J2H	91	75	16	17.6	34	24	10	29.4
21J7H	72	65	7	9.7	30	30	0	0
22J2C	53	50	3	5.7	29	27	2	6.9
22J7C	67	56	11	16.4	34	27	7	20.6
22J2H	75	69	6	8.0	53	50	3	5.7
22J7H	210	189	21	10.0	152	128	24	15.8
21K2C	47	40	7	14.9	51	47	4	7.8
21K7C	52	37	15	28.8	54	53	1	1.9
21K2H	25	19	6	24.0	57	48	9	15.8
21K7H	28	20	8	28.6	74	65	9	12.2
22K2C	60	50	10	16.7	55	38	17	30.9
22K7C	50	48	2	4.0	35	33	2	5.7
22K2H	44	43	1	2.3	42	36	6	14.3
22K7H	112	103	9	8.0	91	89	2	2.2

TABLE 9

The table shows the total number of specimens, the number of Ab positive specimens, the number of Ab negative specimens, and the percentage positive Ab per area, for two vole propagation cycles. The first cycle includes the capture seasons fall 79 - fall 82. The second cycle includes the seasons spring 83 - spring 86. The results from capture season fall 87 were not used.

AREA	CYCLE 1				CYCLE 2			
	TOT	NEG	POS	%POS	TOT	NEG	POS	%POS
21J2C	153	114	39	25.5	162	131	31	19.1
21J7C	129	95	34	26.4	139	116	23	16.5
21J2H	91	66	25	27.5	34	25	9	26.5
21J7H	72	63	9	12.5	30	30	0	0
22J2C	53	46	7	13.2	29	25	4	13.8
22J7C	67	47	20	29.9	34	30	4	11.8
22J2H	75	55	20	26.7	53	42	11	20.8
22J7H	210	168	42	20.0	152	124	28	18.4
21K2C	47	37	10	21.3	51	43	8	15.7
21K7C	51	24	27	52.9	54	52	2	3.7
21K2H	25	25	0	0	57	47	10	17.5
21K7H	28	22	6	21.4	74	69	5	6.8
22K2C	60	33	27	45.0	55	46	9	16.4
22K7C	50	42	8	16.0	35	32	3	8.6
22K2H	44	32	12	27.3	42	35	7	16.7
22K7H	112	91	21	18.8	91	89	2	2.2

TABLE 10

The table shows the total number of specimens, the number of Ag negative specimens, the number of Ag positive specimens, and the percentage Ag positive specimens per area and coordinate, for two vole propagation cycles. The first cycle includes the capture seasons fall 79 - fall 82. The second cycle includes the seasons spring 83 - spring 86. The results from capture season fall 87 were not used.

AREA	COORD	CYCLE 1				CYCLE 2			
		TOT	NEG	POS	%POS	TOT	NEG	POS	%POS
21J2C	1212	45	41	4	8.9	48	37	11	22.9
	1237	4	4	0	0	6	4	2	33.3
	3712	54	40	14	25.9	35	33	2	5.7
	3737	50	46	4	8.0	73	62	11	15.1
21J7C	1212	36	34	2	5.6	43	31	12	27.9
	1237	42	39	3	7.1	12	9	3	25.0
	3712	25	22	3	12.0	35	28	7	20.0
	3737	26	26	0	0	49	32	17	34.7
21J2H	1212	2	2	0	0	13	11	2	15.4
	1237	0	0	0	0	0	0	0	-
	3712	76	61	15	19.7	7	1	6	85.7
	3737	13	12	1	7.7	14	12	2	14.3
21J7H	1212	19	17	2	10.5	12	12	0	0
	1237	16	13	3	18.8	7	7	0	0
	3712	33	31	2	6.1	9	9	0	0
	3737	4	4	0	0	2	2	0	0
22J2C	1212	2	2	0	0	4	4	0	0
	1237	7	7	0	0	4	3	1	25.0
	3712	26	25	1	3.8	8	8	0	0
	3737	18	16	2	11.1	13	12	1	7.7
22J7C	1212	43	35	8	18.6	26	24	2	7.7
	1237	7	6	1	14.3	1	1	0	0
	3712	17	15	2	11.8	7	2	5	71.4
	3737	0	0	0	-	0	0	0	-
22J2H	1212	0	0	0	-	0	0	0	-
	1237	11	9	2	18.2	1	1	0	0
	3712	37	33	4	10.8	36	33	3	8.3
	3737	27	27	0	0	16	16	0	0
22J7H	1212	61	58	3	4.9	32	29	3	9.4
	1237	48	44	4	8.3	43	35	8	18.6
	3712	61	47	14	23.0	43	34	9	20.9
	3737	40	40	0	0	34	30	4	11.8
21K2C	1212	9	7	2	22.2	0	0	0	-
	1237	11	10	1	9.1	16	16	0	0
	3712	27	23	4	14.8	27	24	3	11.1
	3737	0	0	0	-	8	7	1	12.5



TABLE 10. CONT

AREA	COORD	CYCLE 1				CYCLE 2			
		TOT	NEG	POS	%POS	TOT	NEG	POS	%POS
21K7C	1212	16	11	5	31.3	14	14	0	0
	1237	4	3	1	25.0	11	11	0	0
	3712	7	7	0	0	6	5	1	16.7
	3737	25	16	9	36.0	23	23	0	0
21K2H	1212	0	0	0	-	0	0	0	-
	1237	6	3	3	50.0	25	23	2	8.0
	3712	9	7	2	22.2	15	10	5	33.3
	3737	0	9	1	10.0	17	15	2	11.8
21K7H	1212	0	0	0	-	2	2	0	0
	1237	10	4	6	60.0	29	26	3	10.3
	3712	1	1	0	0	18	14	4	22.2
	3737	17	15	2	11.8	25	23	2	8.0
22K2C	1212	4	4	0	0	2	1	1	50.0
	1237	19	15	4	21.1	10	7	3	30.0
	3712	2	2	0	0	2	2	0	0
	3737	35	29	6	17.1	41	28	13	31.7
22K7C	1212	6	6	0	0	3	3	0	0
	1237	32	30	2	6.3	31	29	2	6.5
	3712	7	7	0	0	1	1	0	0
	3737	5	5	0	0	0	0	0	-
22K2H	1212	33	32	1	3.0	33	27	6	18.2
	1237	0	0	0	-	0	0	0	-
	3712	0	0	0	-	0	0	0	-
	3737	11	11	0	0	9	9	0	0
22K7H	1212	35	35	0	0	25	24	1	4.0
	1237	47	38	9	19.1	35	34	1	2.9
	3712	0	0	0	-	0	0	0	-
	3737	30	30	0	0	31	31	0	0

FIGURE 1

## PCT AG POS IN DIFFERENT VOLE SPECIES

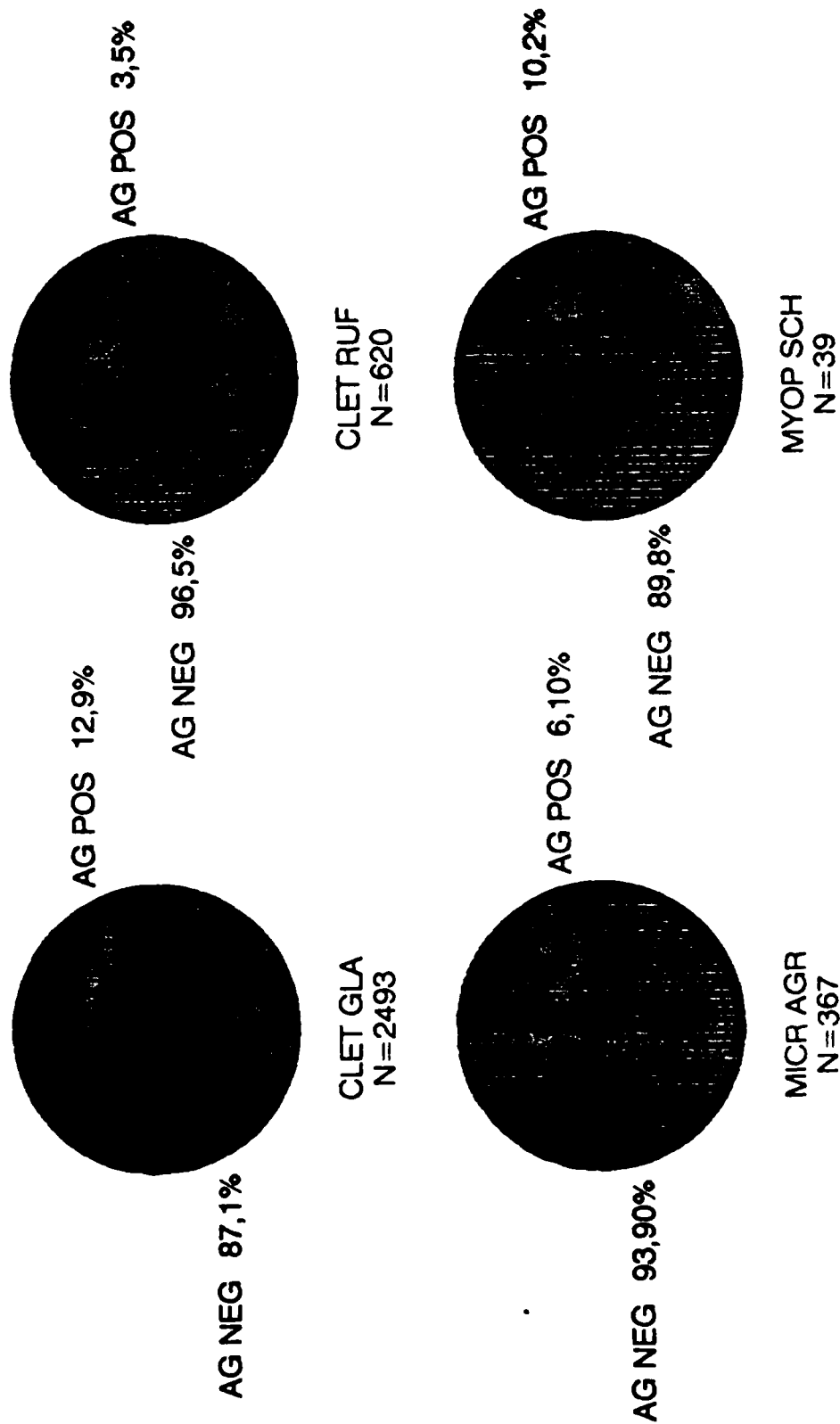


FIGURE 2

## PCT AB POS IN DIFFERENT VOLE SPECIES

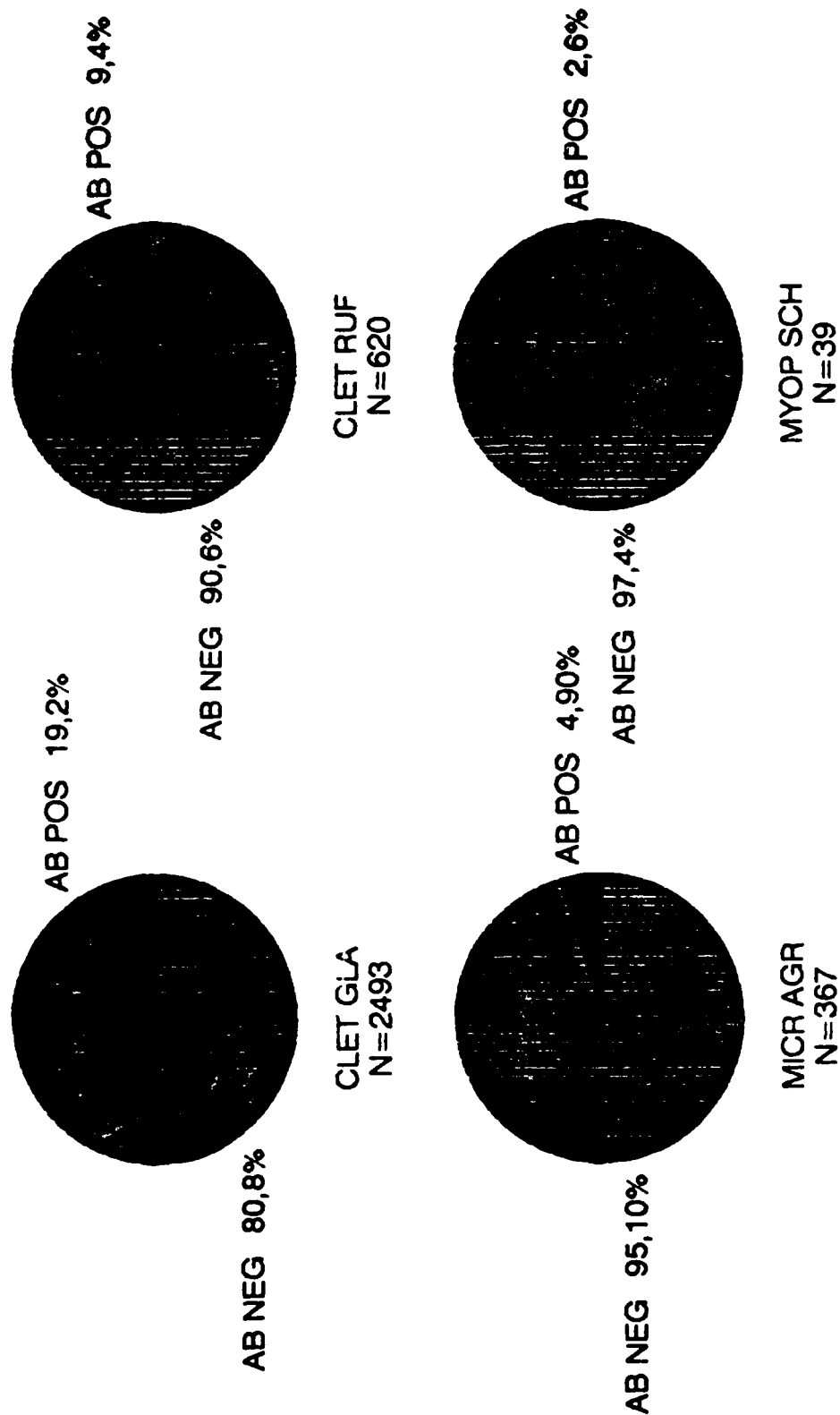


FIGURE 3

# CLET GLA

PCT AG POS AND NUMBERS, PER SEASON AND YEAR

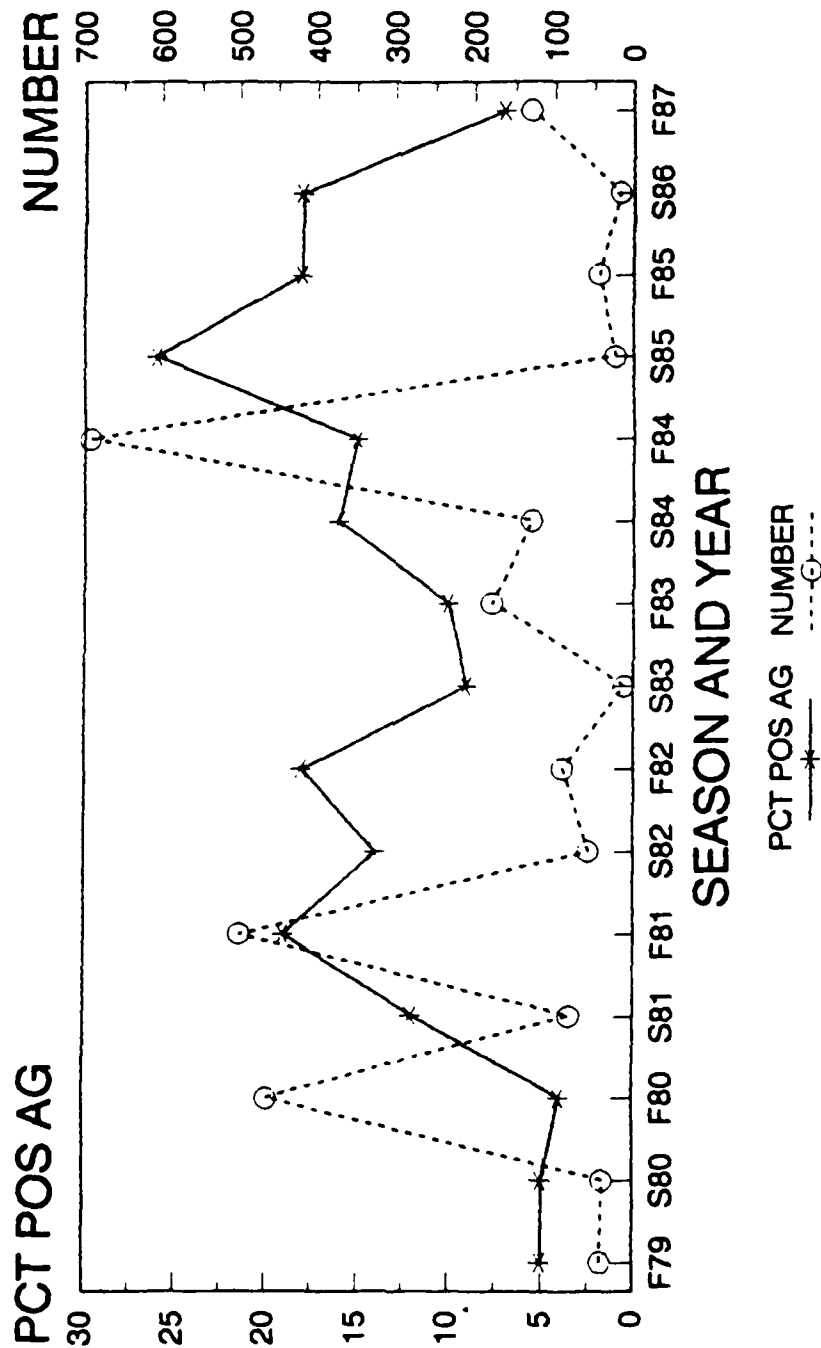


FIGURE 4

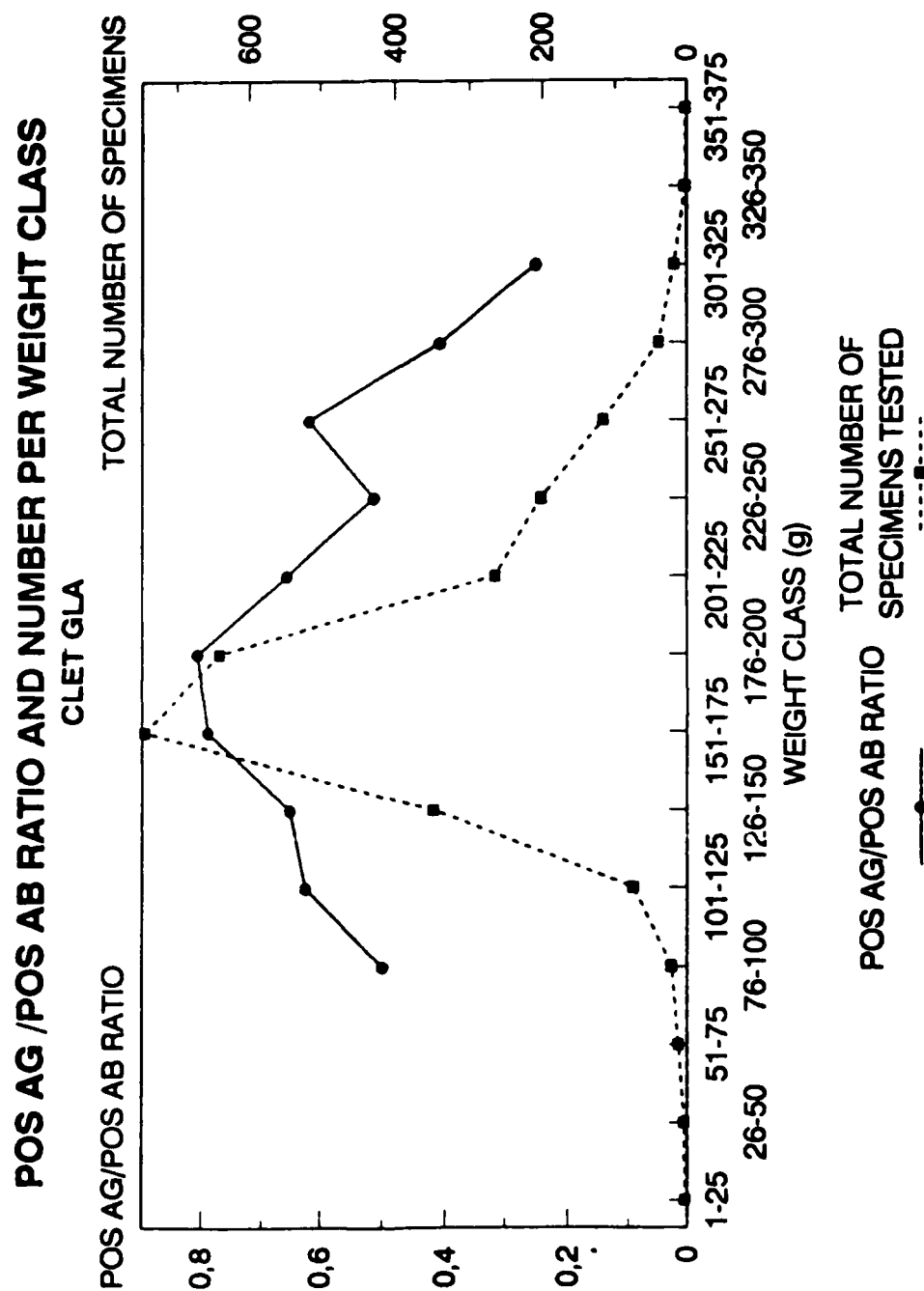


FIGURE 5

# NUMBER OF POS AB PER WEIGHT CLASS

CLET GLA

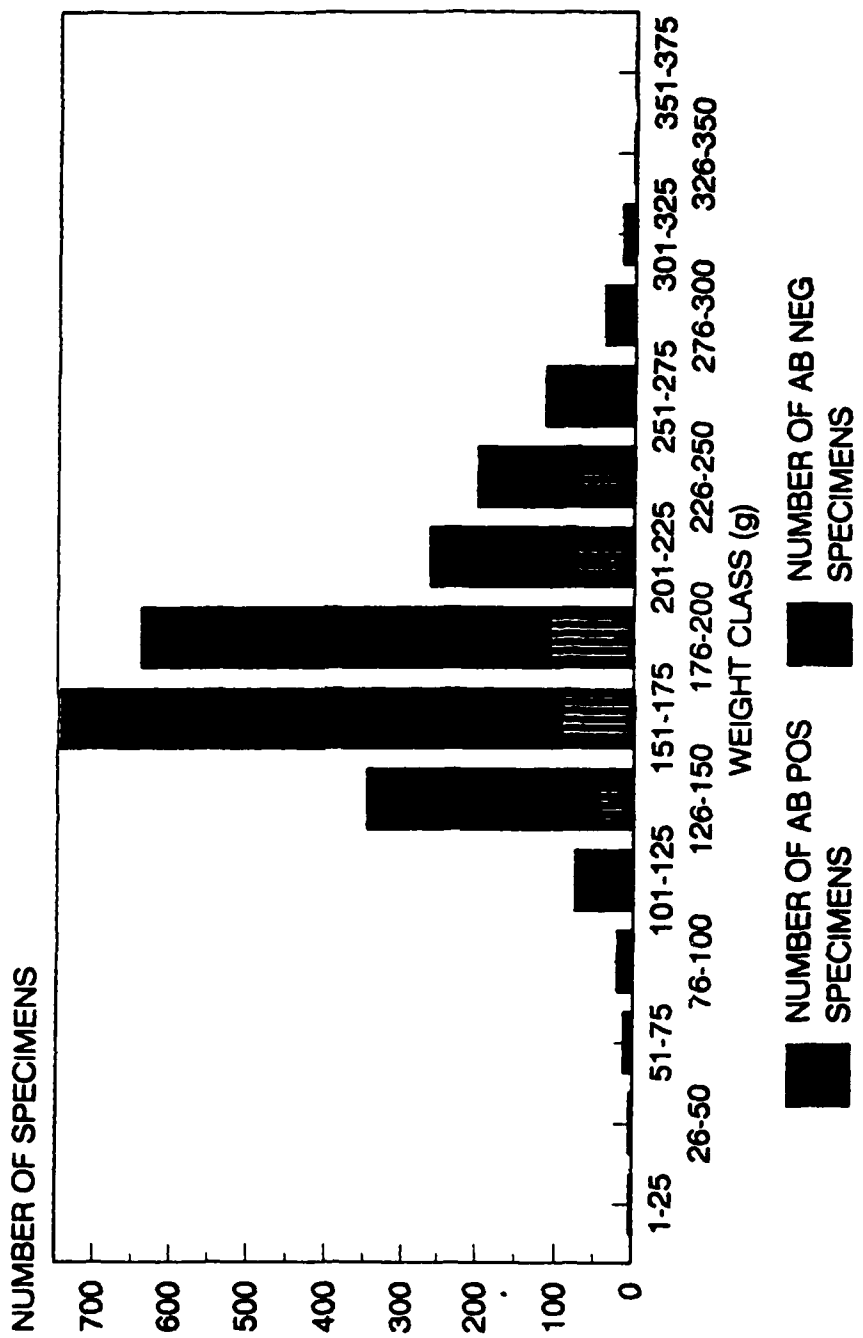


FIGURE 6

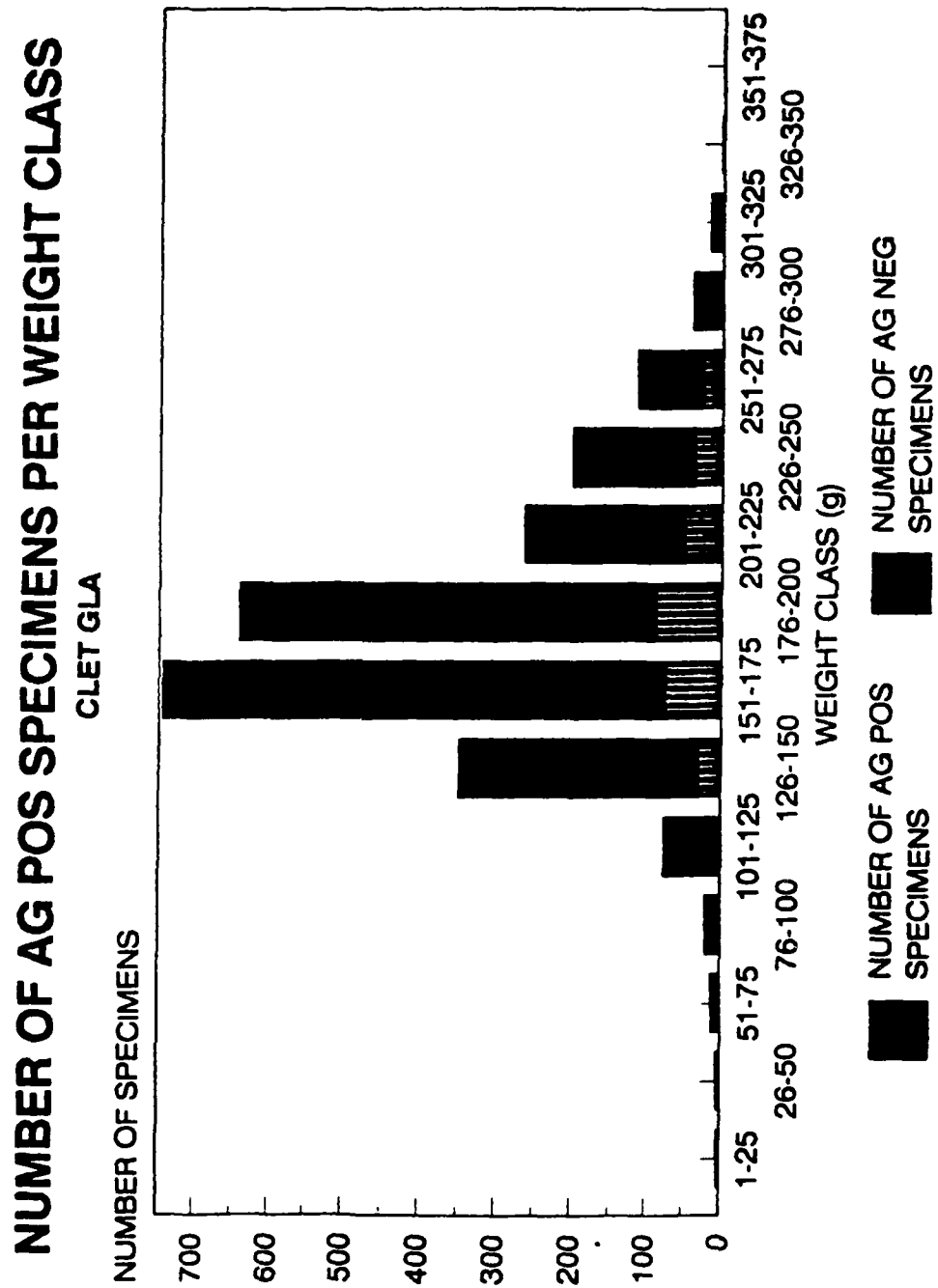


FIGURE 7

# PCT POS AB PER WEIGHT CLASS

CLET GLA

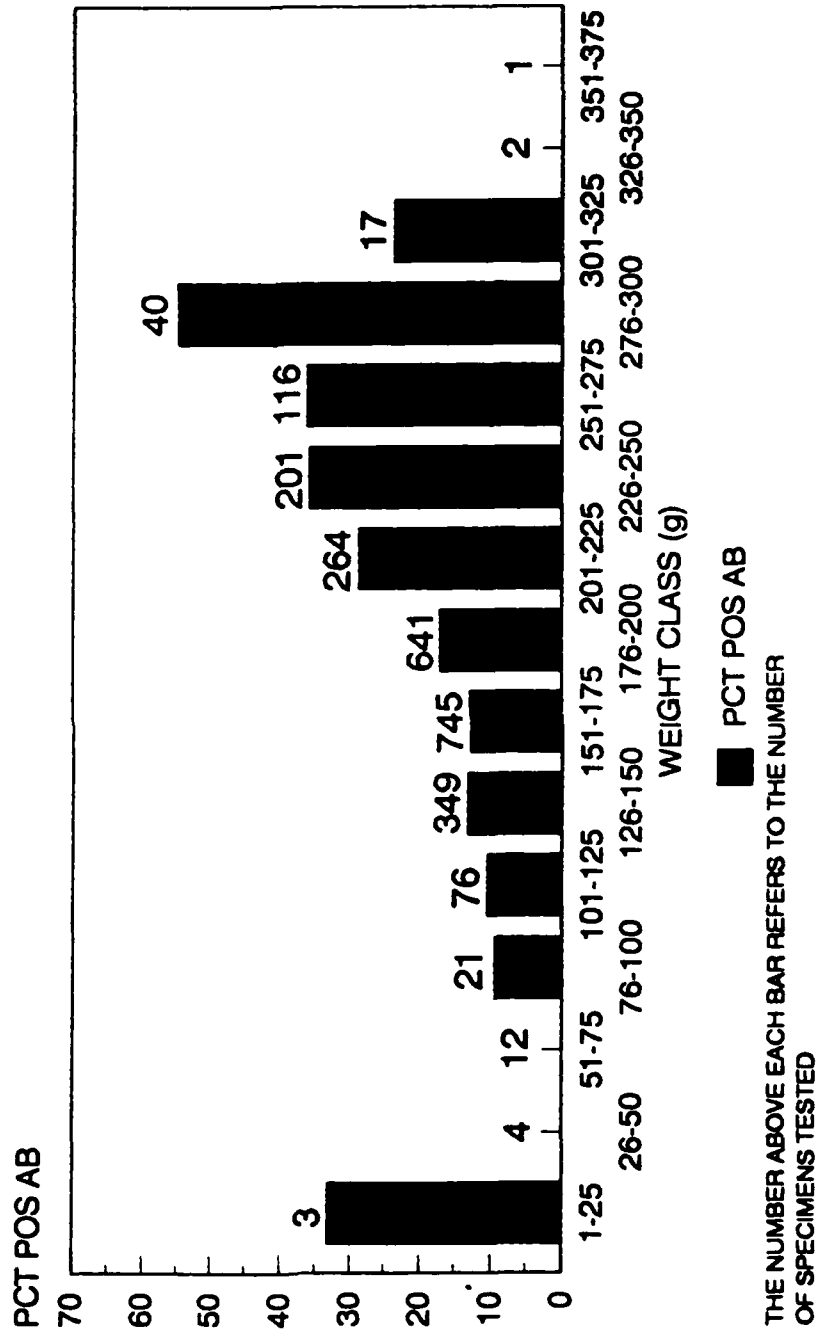




FIGURE 8

# PCT POS AG PER WEIGHT CLASS

CLET GLA

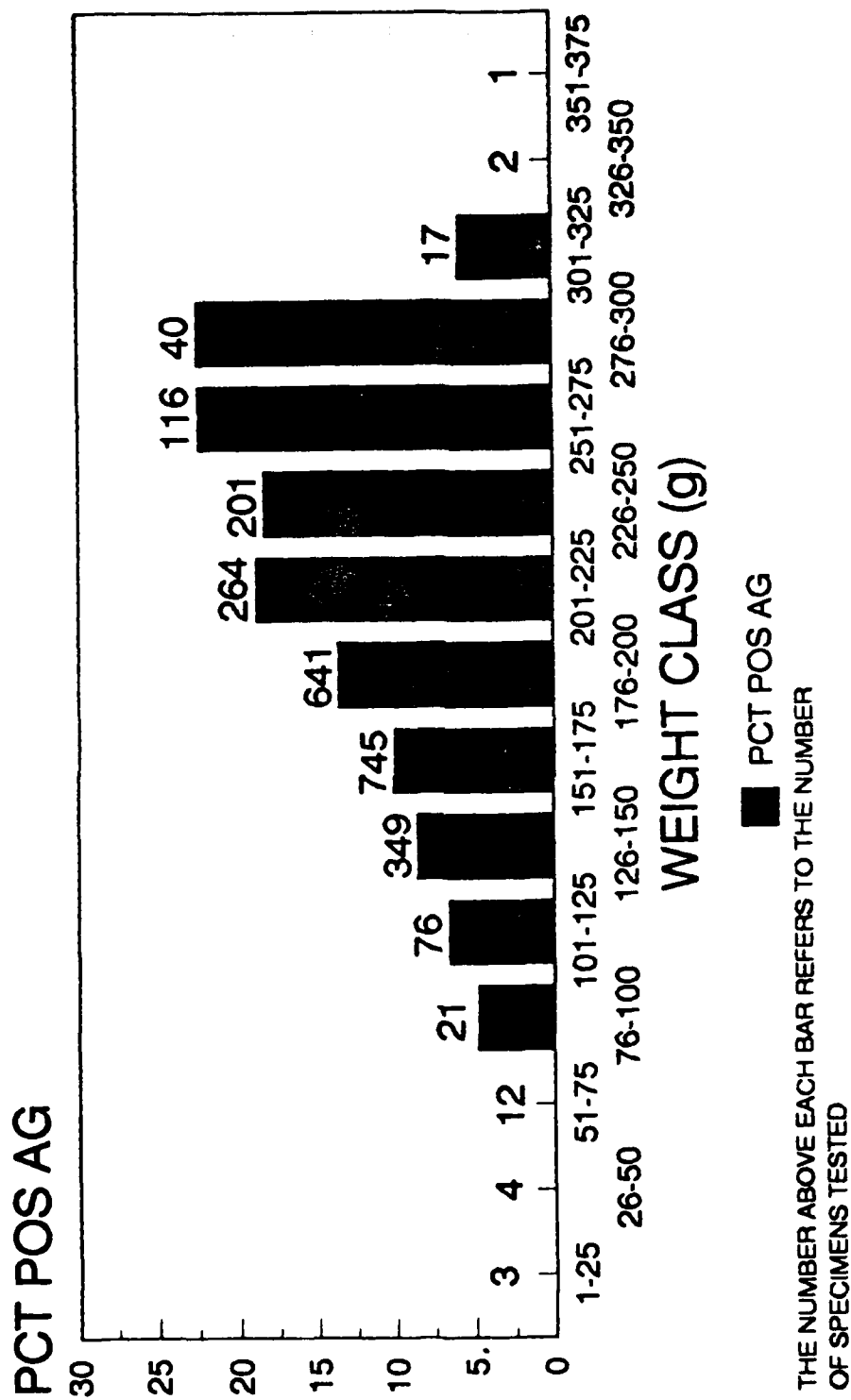


FIGURE 9

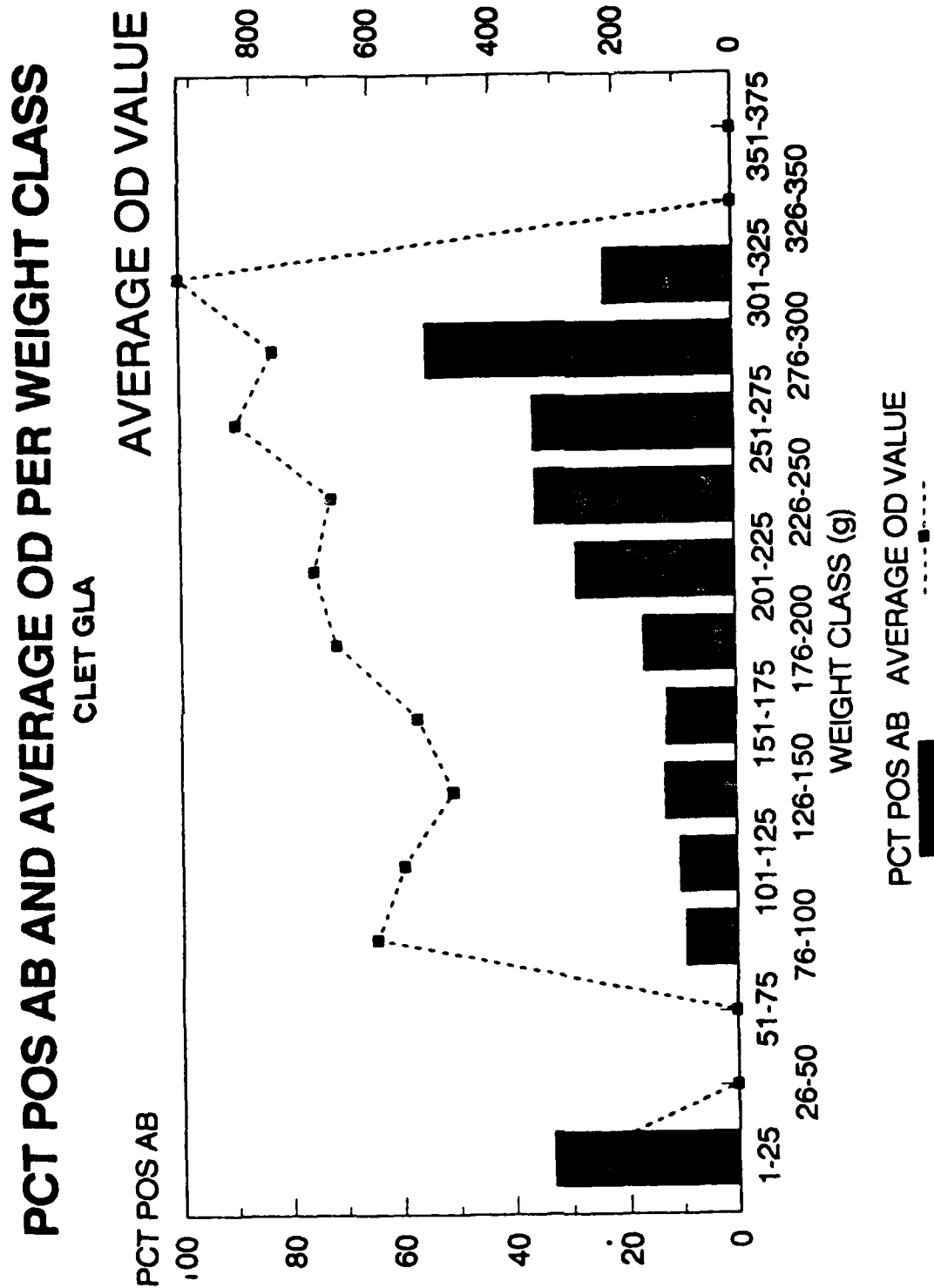


FIGURE 10

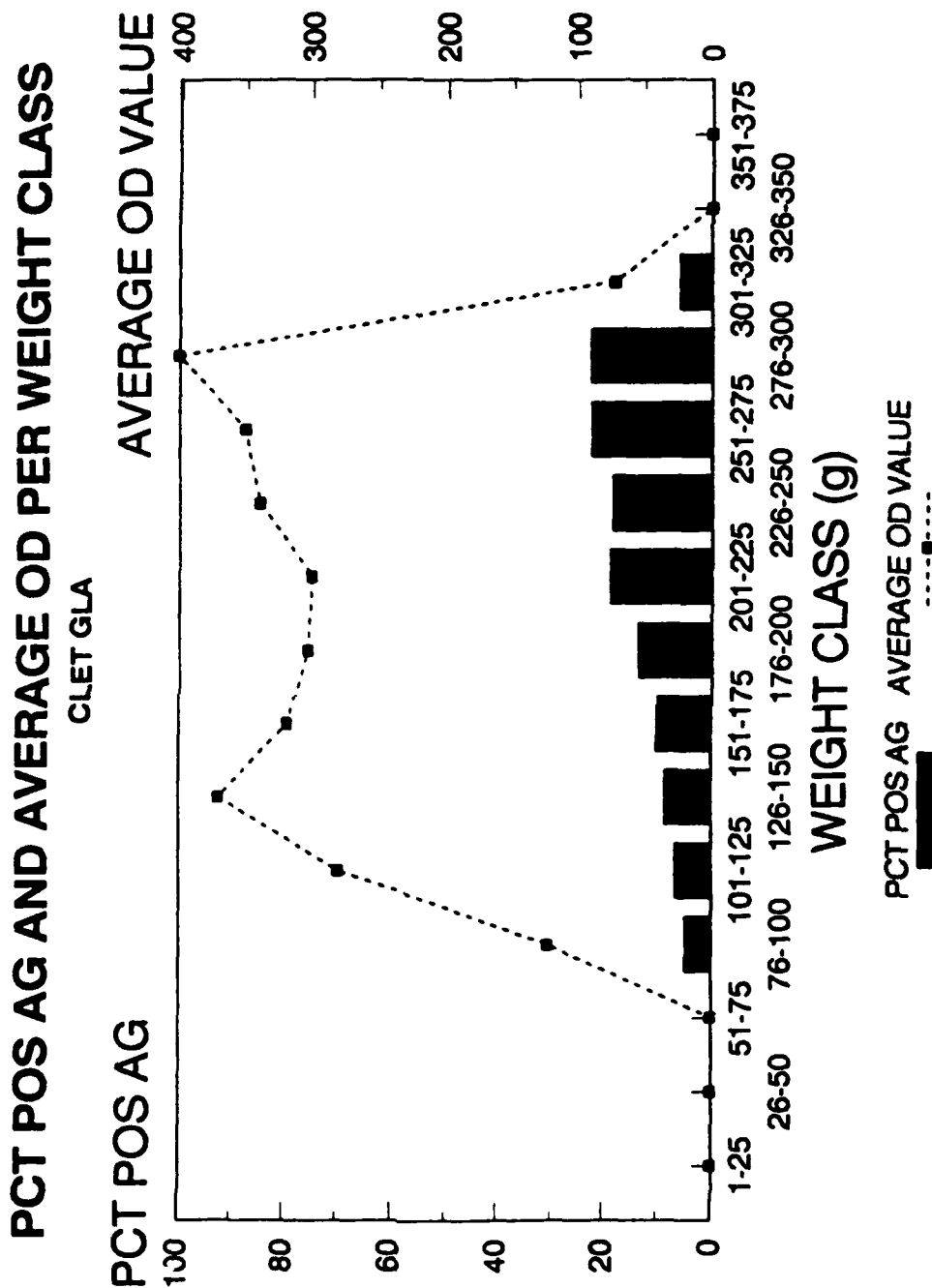


FIGURE 11

# NUMBER OF SPECIMENS PER AREA AND CYCLE

CLET GLA

TOTAL NUMBER OF SPECIMENS

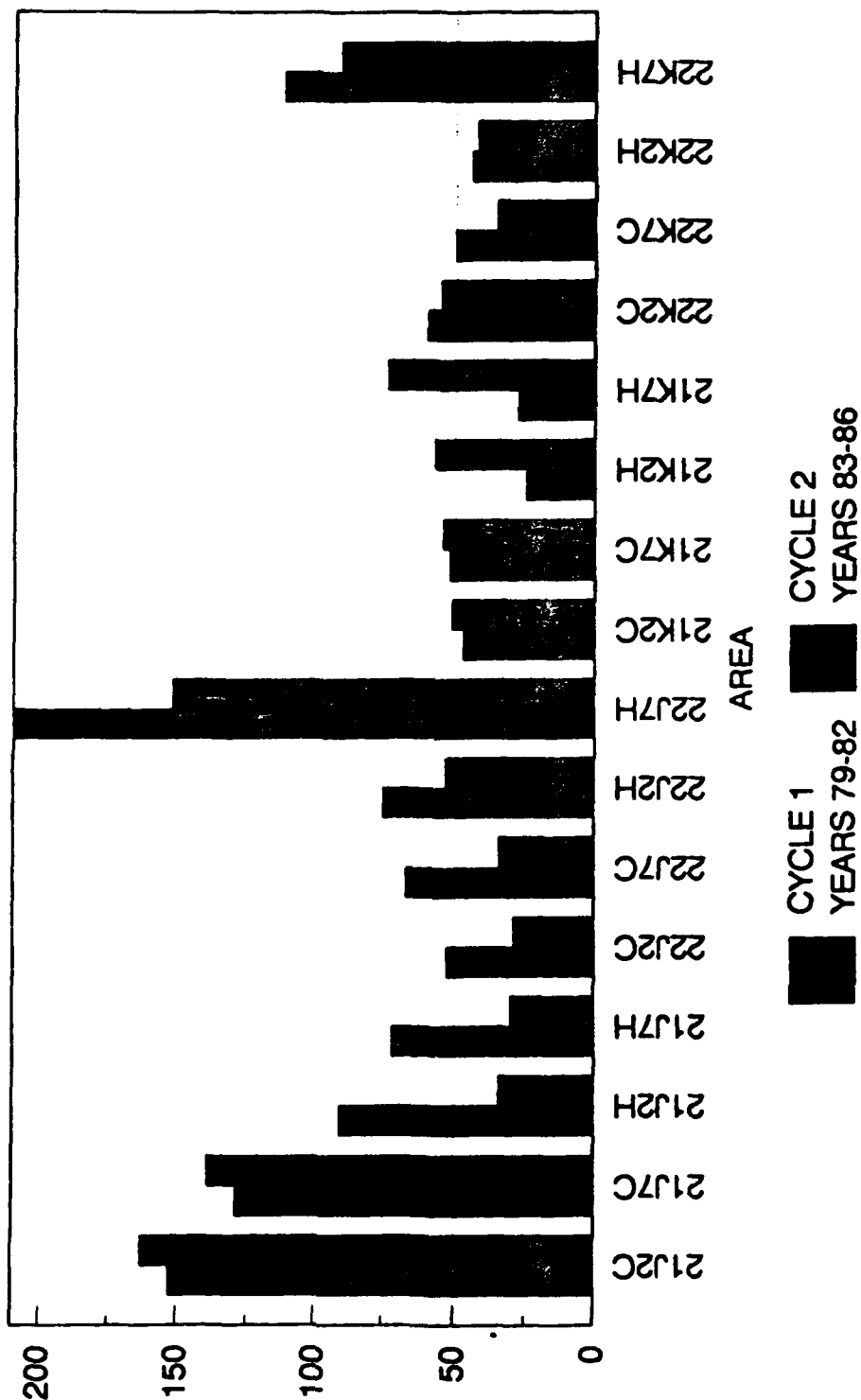


FIGURE 12

# PCT AB POS SPECIMENS PER AREA CLET GLA

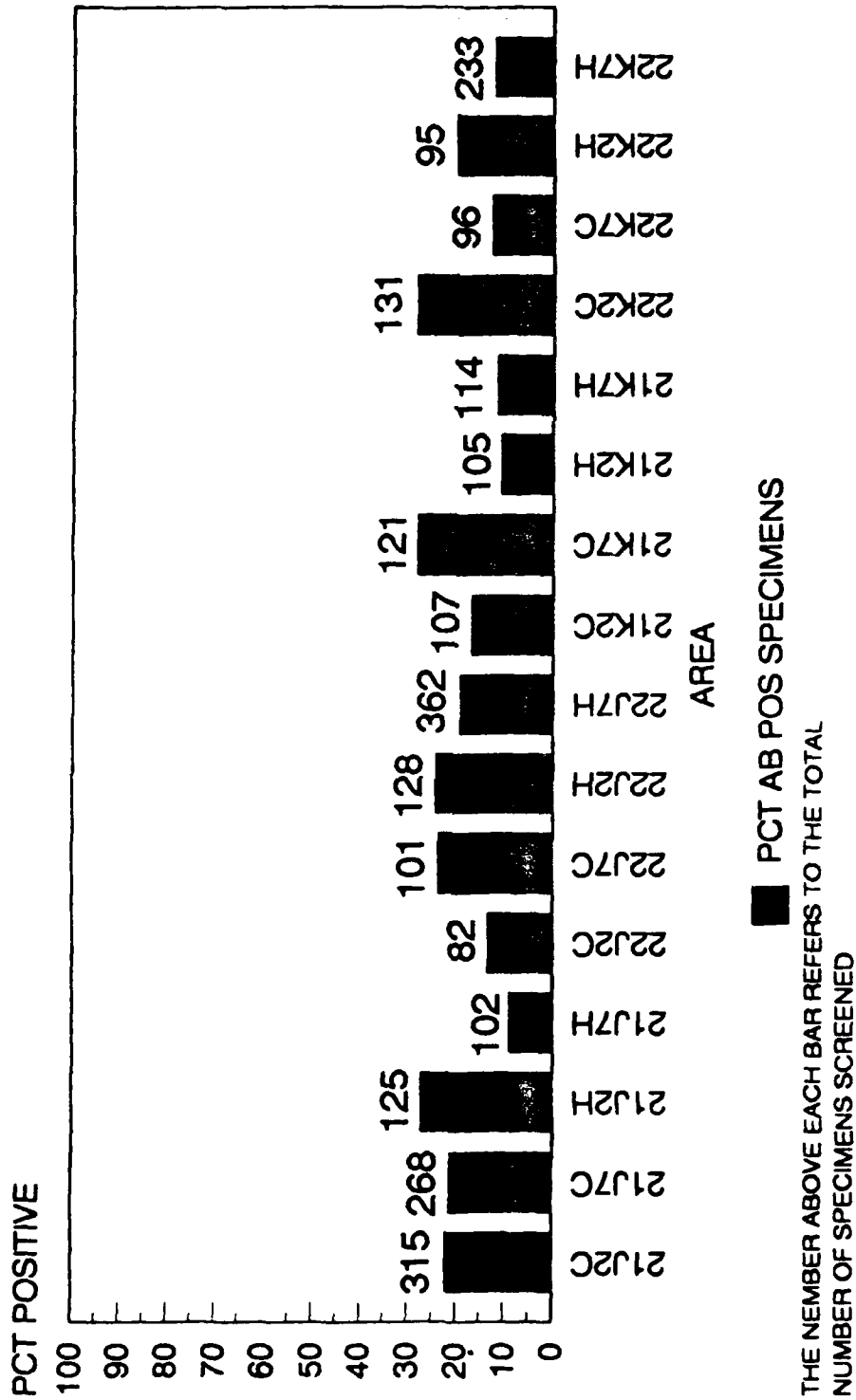


FIGURE 13

# NUMBER OF AG POS/NEG PER AREA

CLET GLA

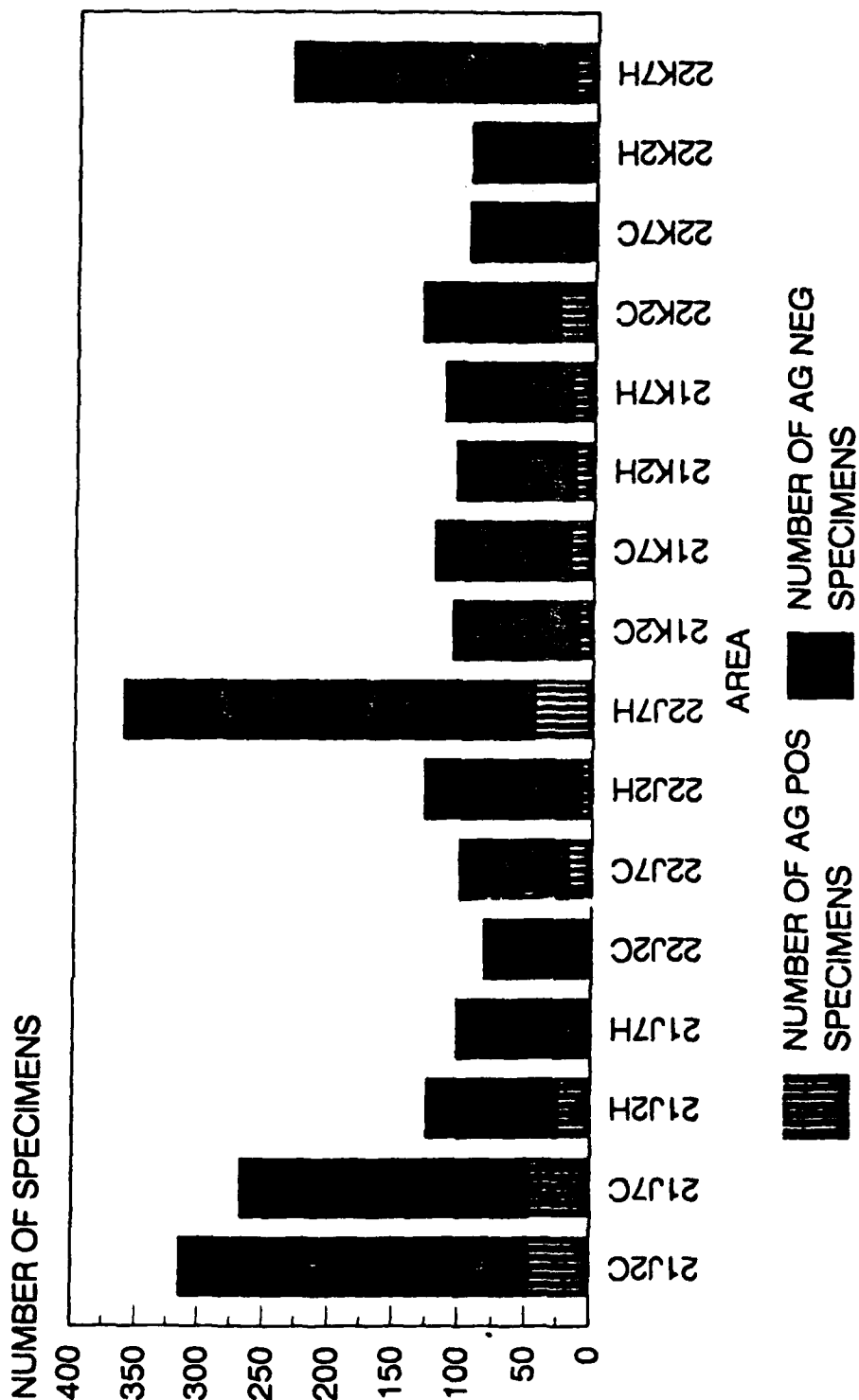


FIGURE 14

# NUMBER OF AB POS/NEG PER AREA

CLET GLA

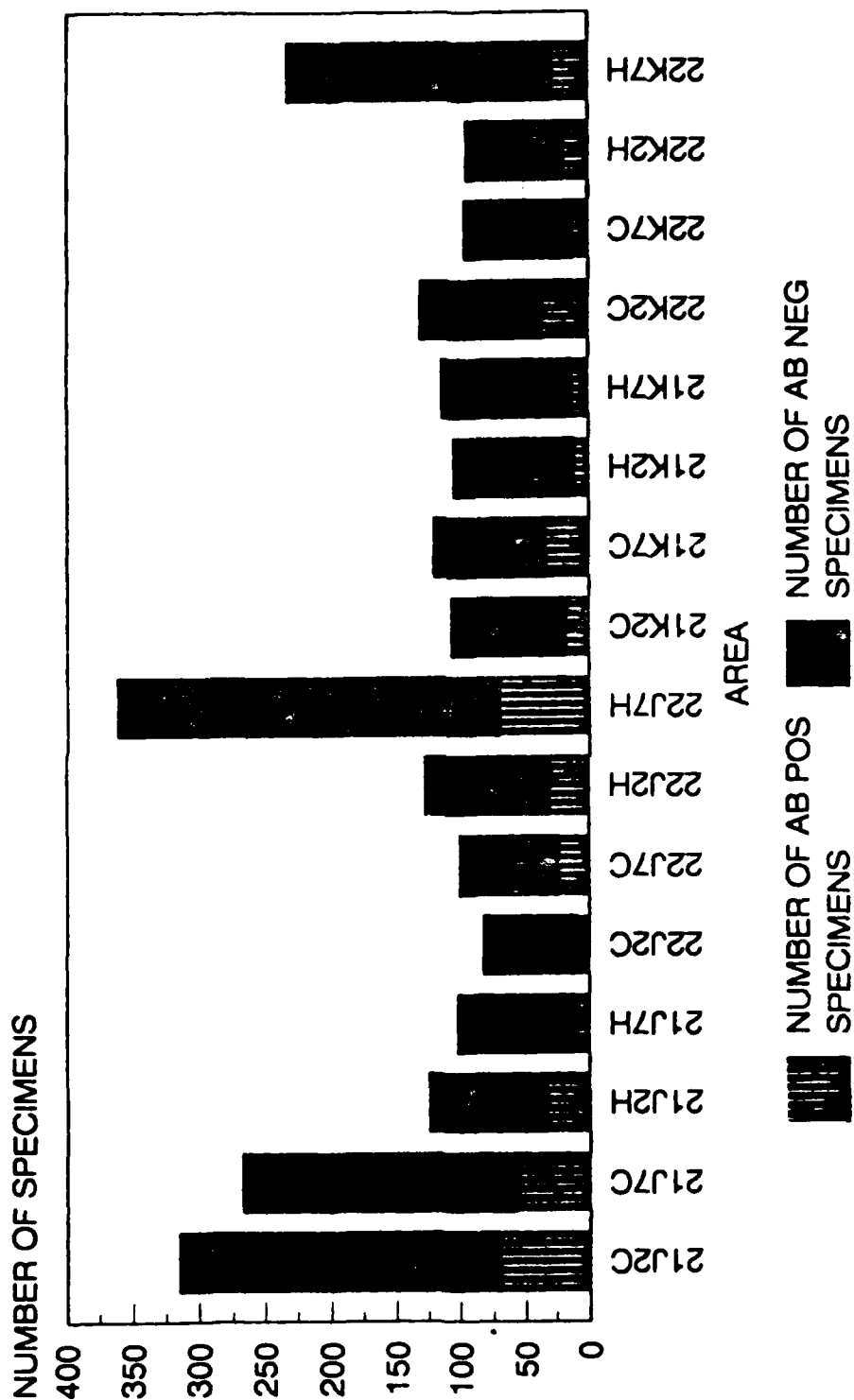
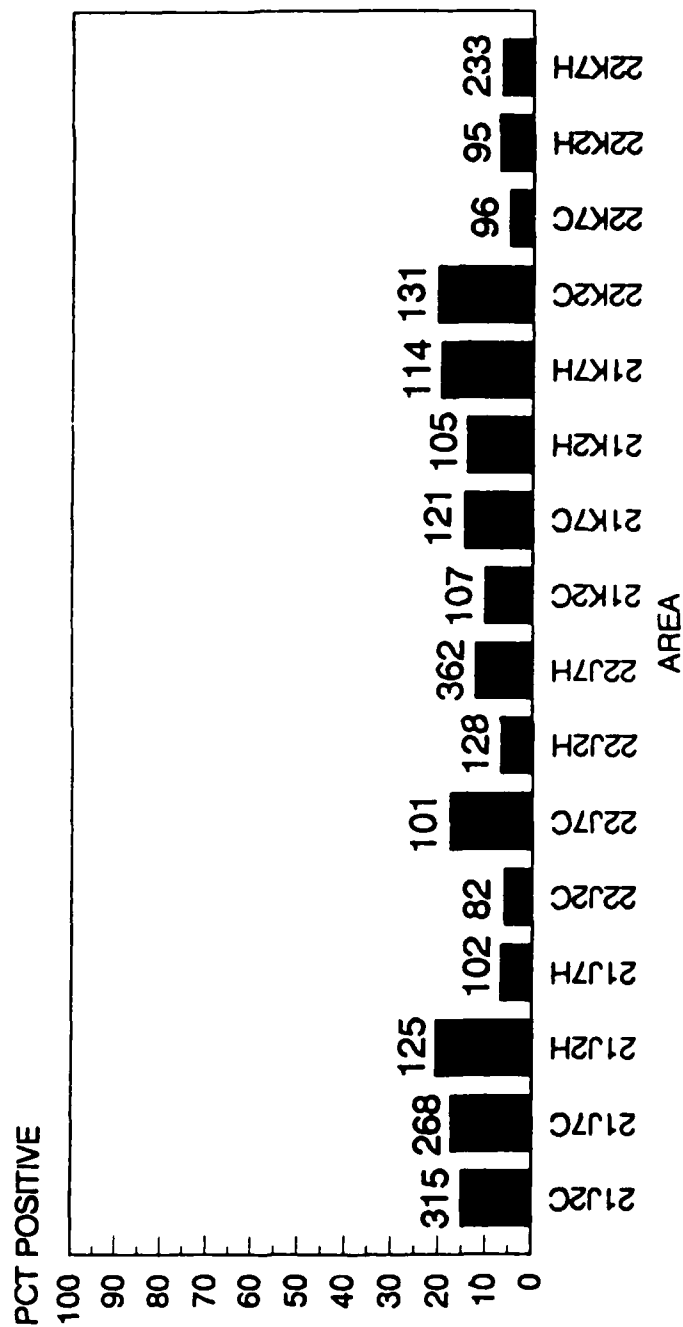


FIGURE 15

# PCT AG POS SPECIMENS PER AREA

CLET GLA



■ PCT AG POS SPECIMENS

THE NUMBER ABOVE EACH BAR REFERS TO THE TOTAL  
NUMBER OF SPECIMENS SCREENED



FIGURE 16

# PCT AG POS SPECIMENS PER AREA AND CYCLE

CLET GLA

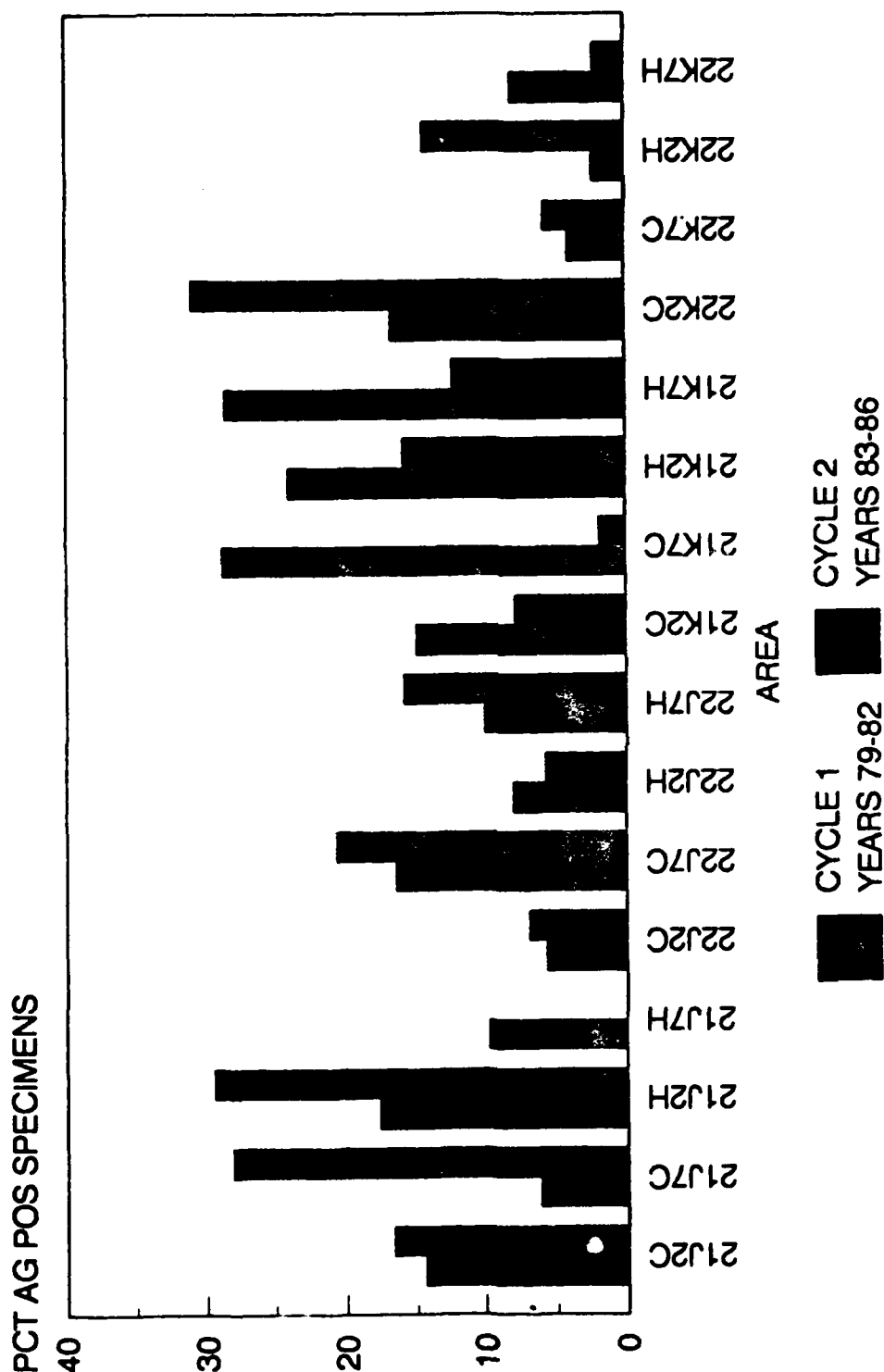


FIGURE 17

# NUMBER OF AG POS SPECIMENS PER AREA AND CYCLE

CLET GLA

NUMBER OF AG POS SPECIMENS

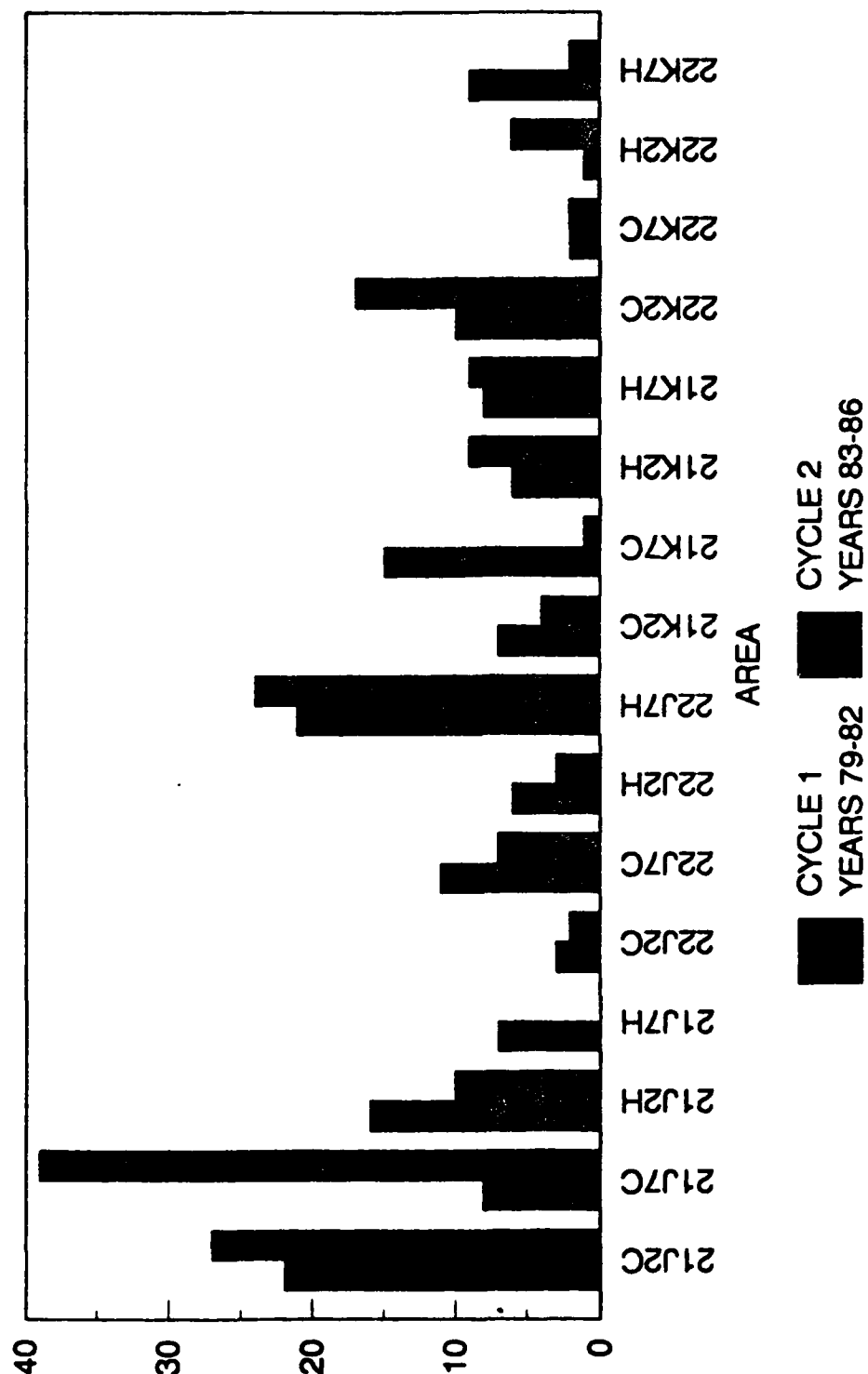


FIGURE 18

# PCT AB POS SPECIMENS PER AREA AND CYCLE

CLET GLA

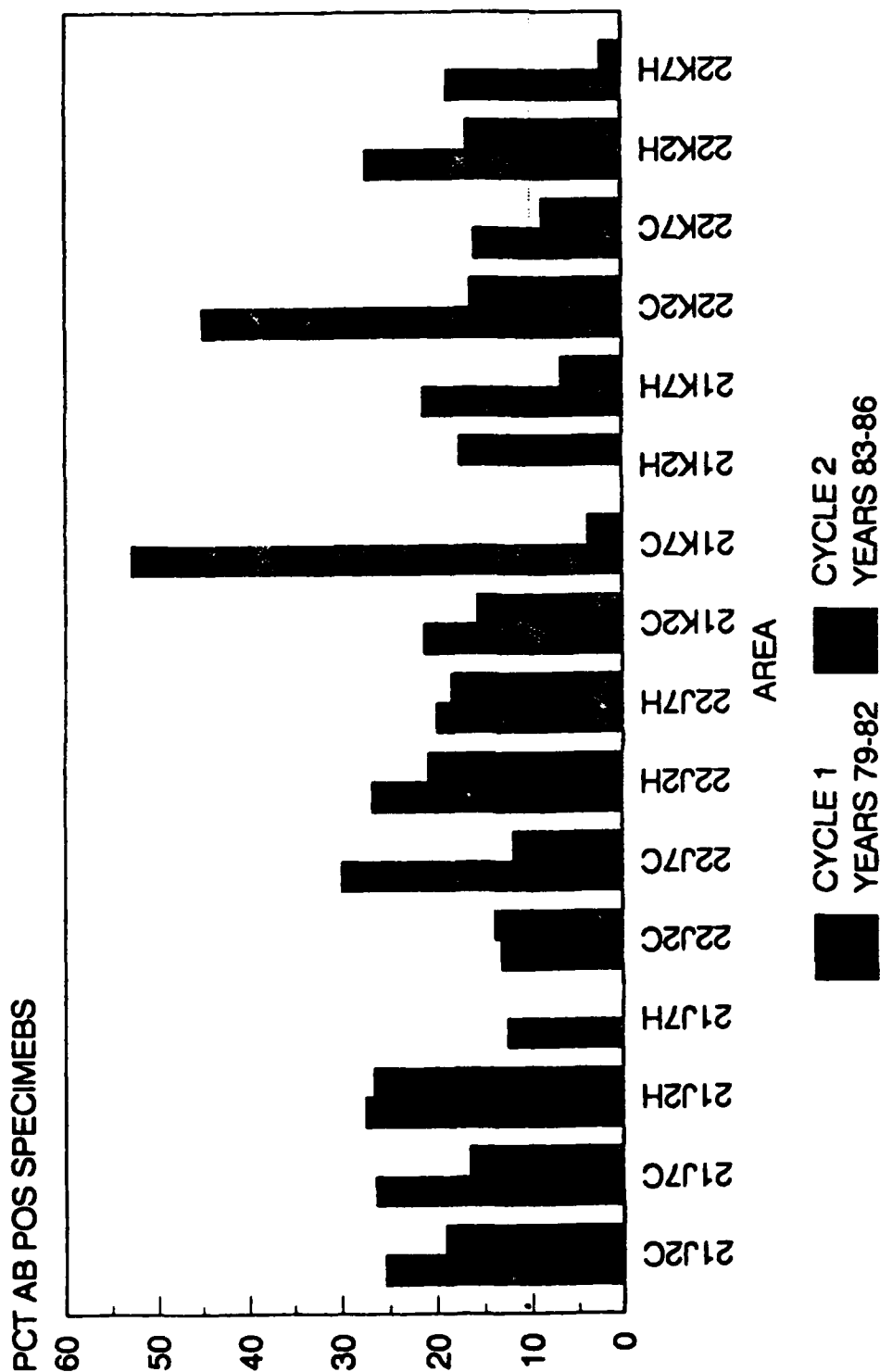


FIGURE 19

# NUMBER OF AB POS SPECIMENS PER AREA AND CYCLE CLET GLA

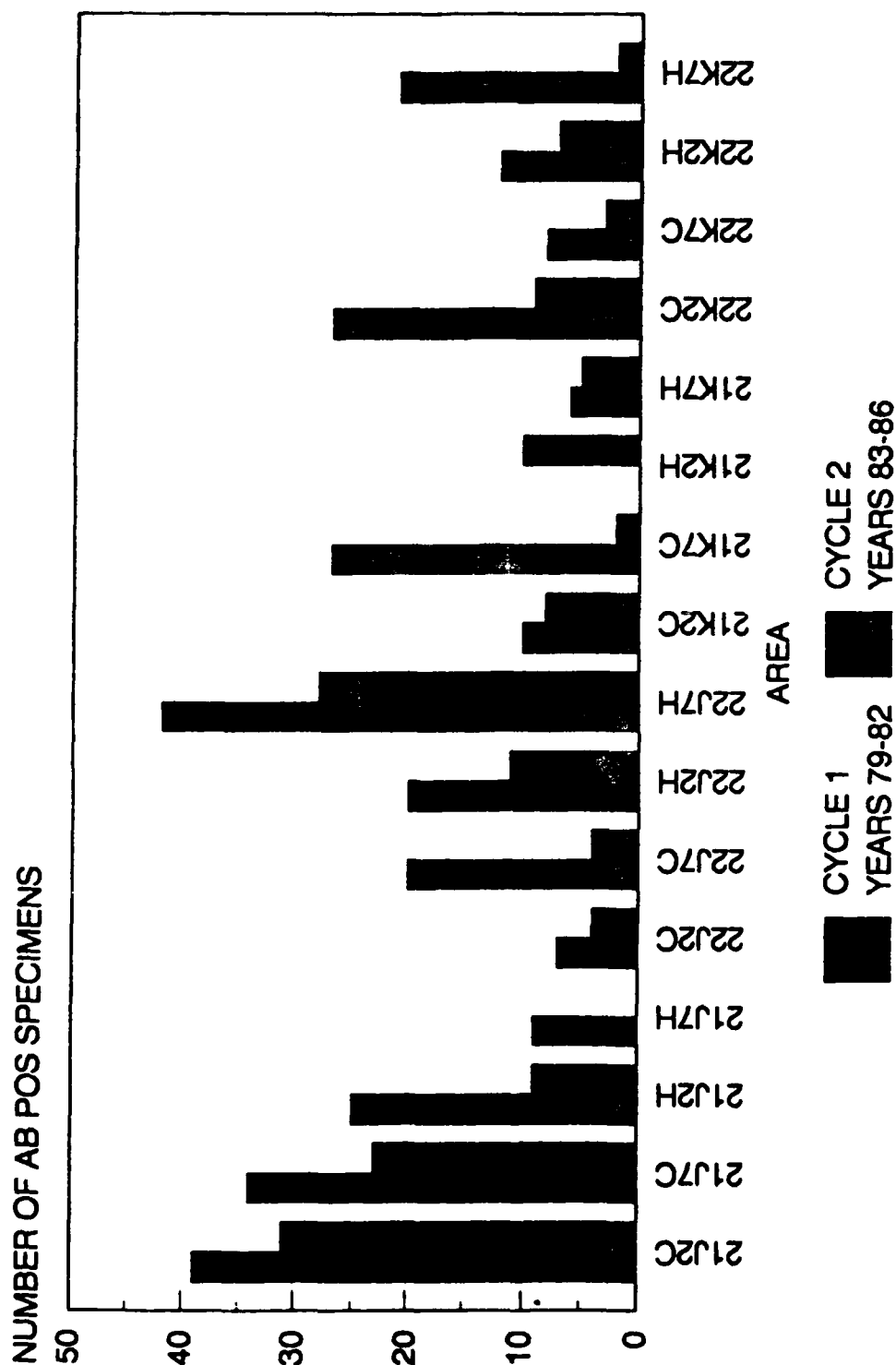
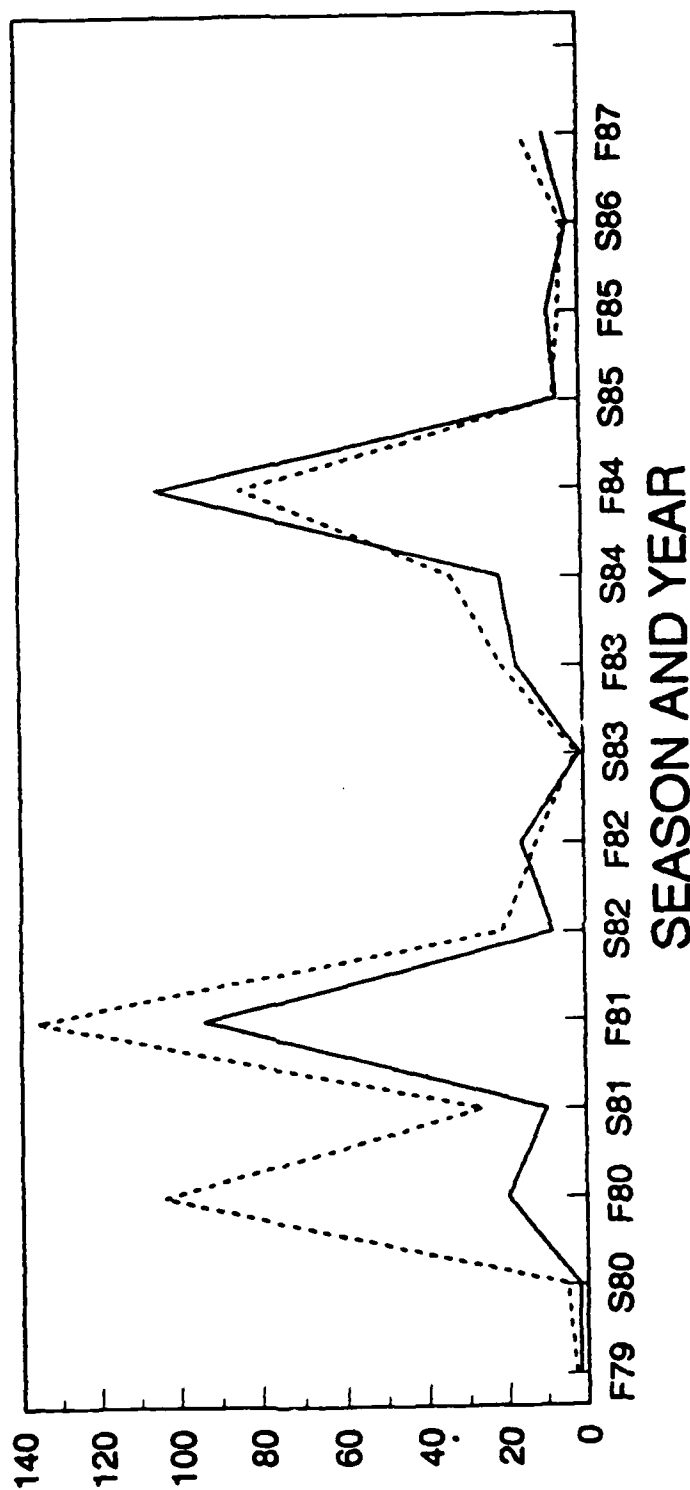


FIGURE 20

# CLET GLA

NO. OF AG AND AB POS SPEC. PER SEASON AND YEAR  
NUMBER OF SPECIMENS



AG POS SPECIMENS AB POS SPECIMENS

S-SPRING F-FALL

FIGURE 21

**TOTAL NUMBER OF SPECIMENS/PCT POSITIVE SPECIMENS  
FOR THE DIFFERENT CAPTURE SEASONS  
AG AND AB**

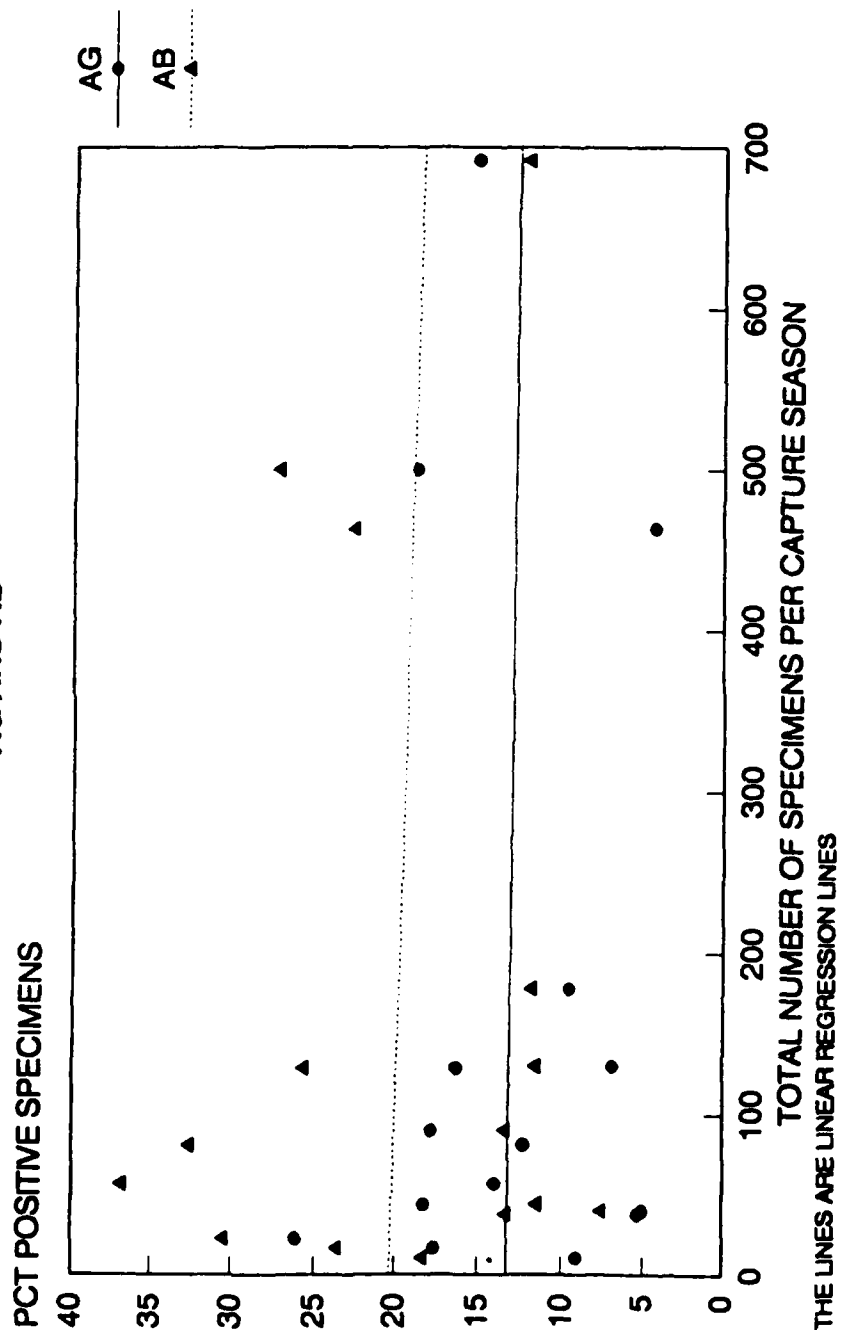


FIGURE 22

# **TOTAL NUMBER OF SPECIMENS/PCT POSITIVE SPECIMENS FOR THE DIFFERENT AREAS AG AND AB**

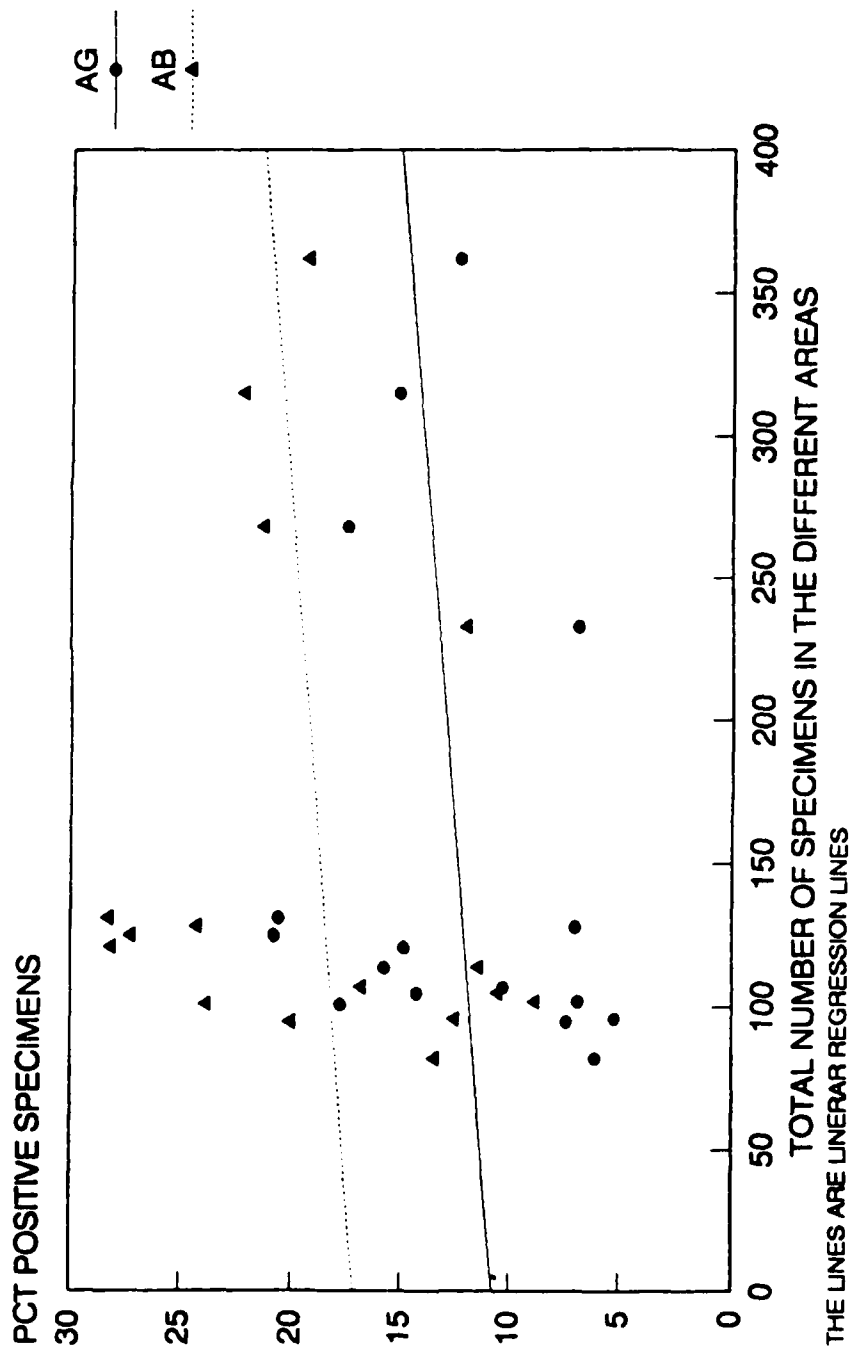


FIGURE 23

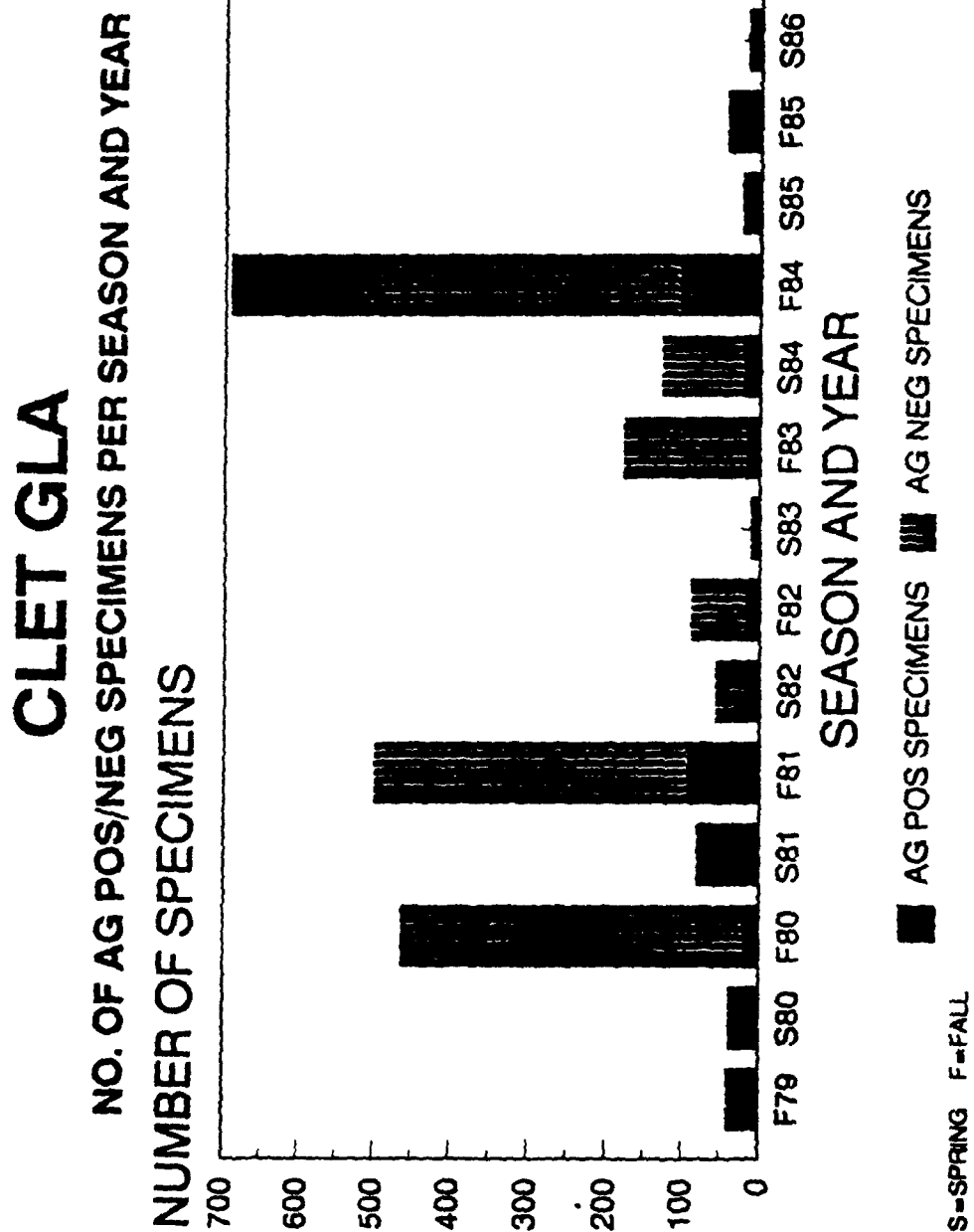




FIGURE 24

# CLET GLA

NO. OF AB POS/NEG SPECIMENS PER SEASON AND YEAR

NUMBER OF SPECIMENS

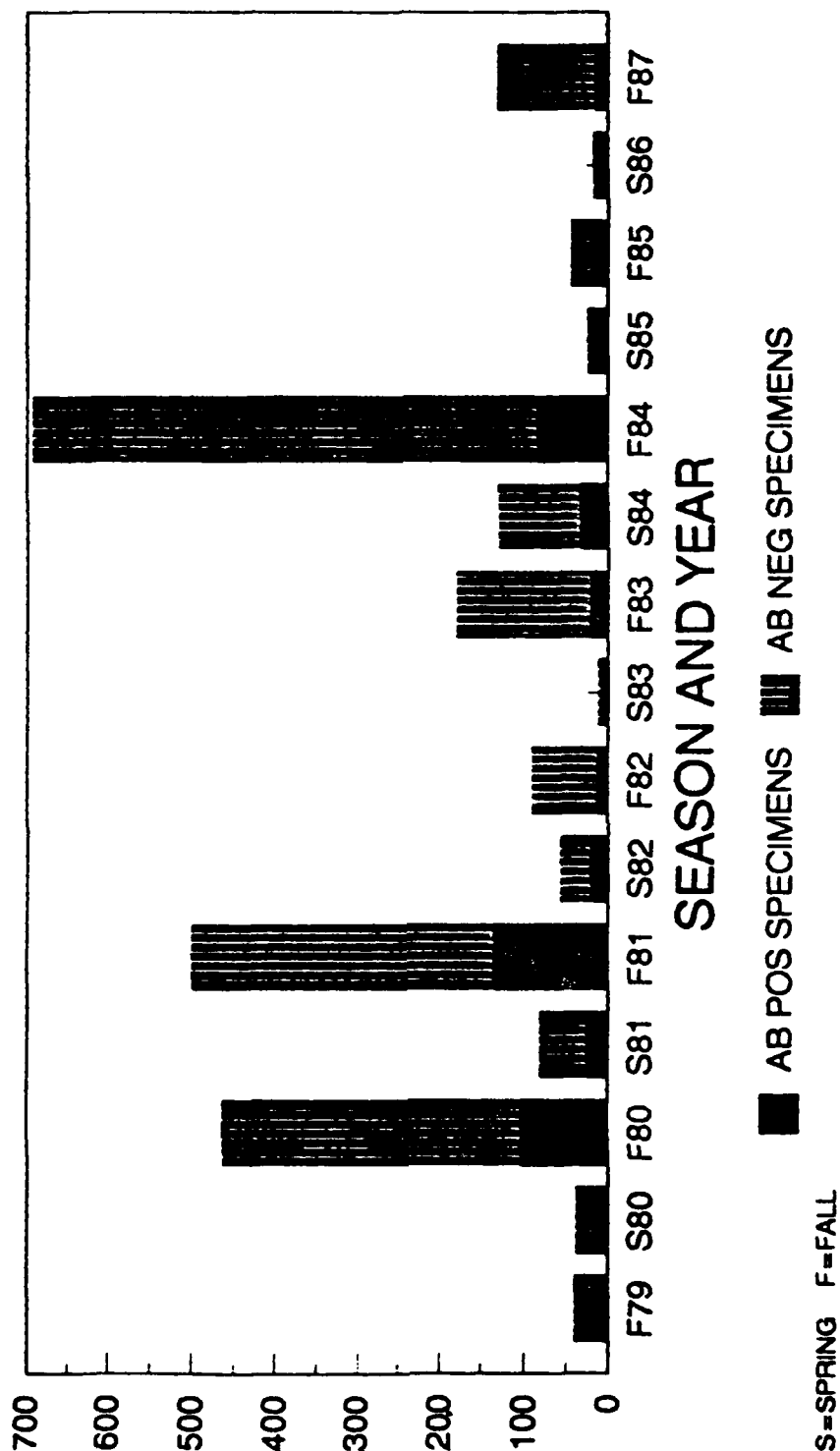


FIGURE 25

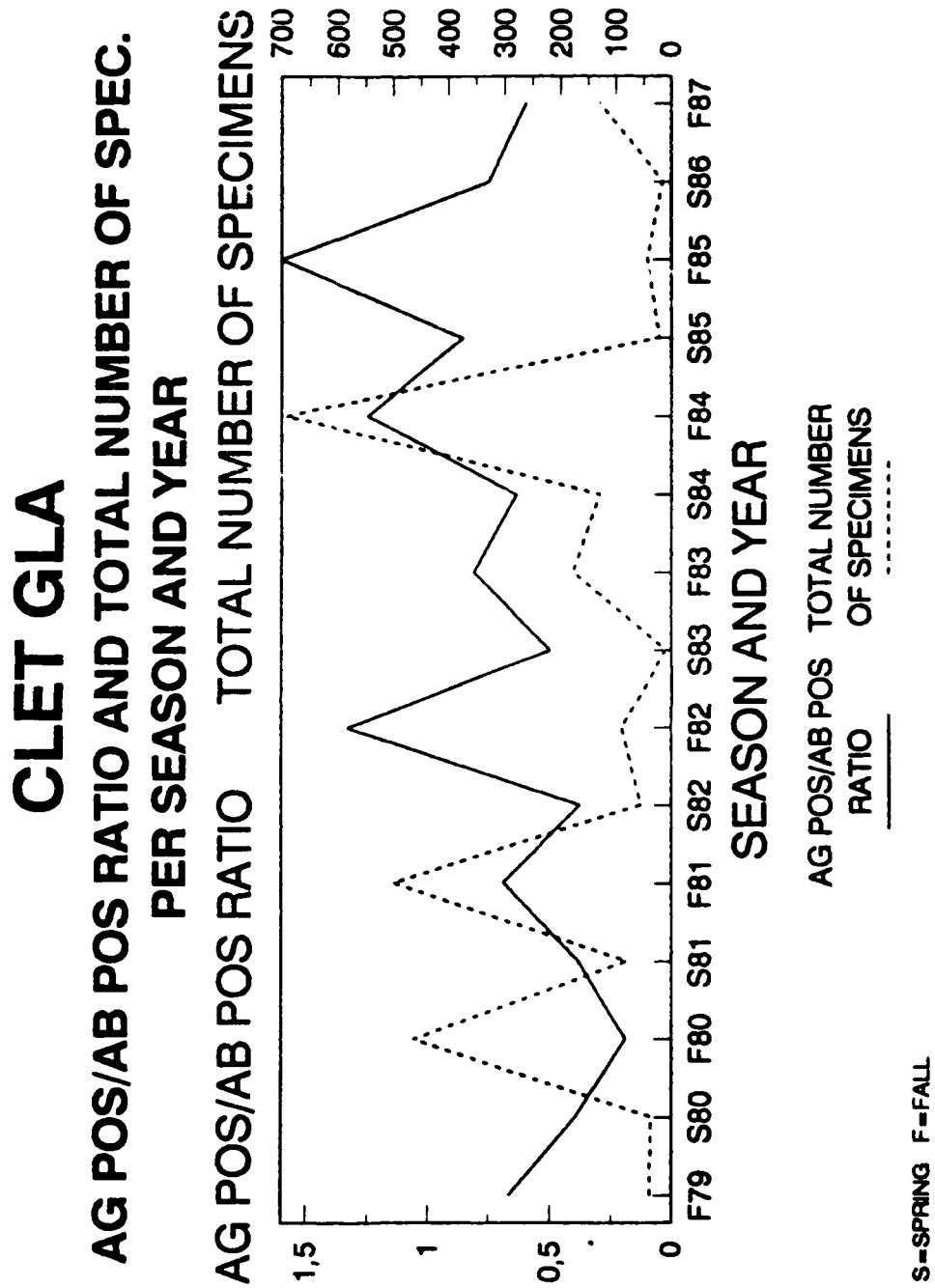


FIGURE 26

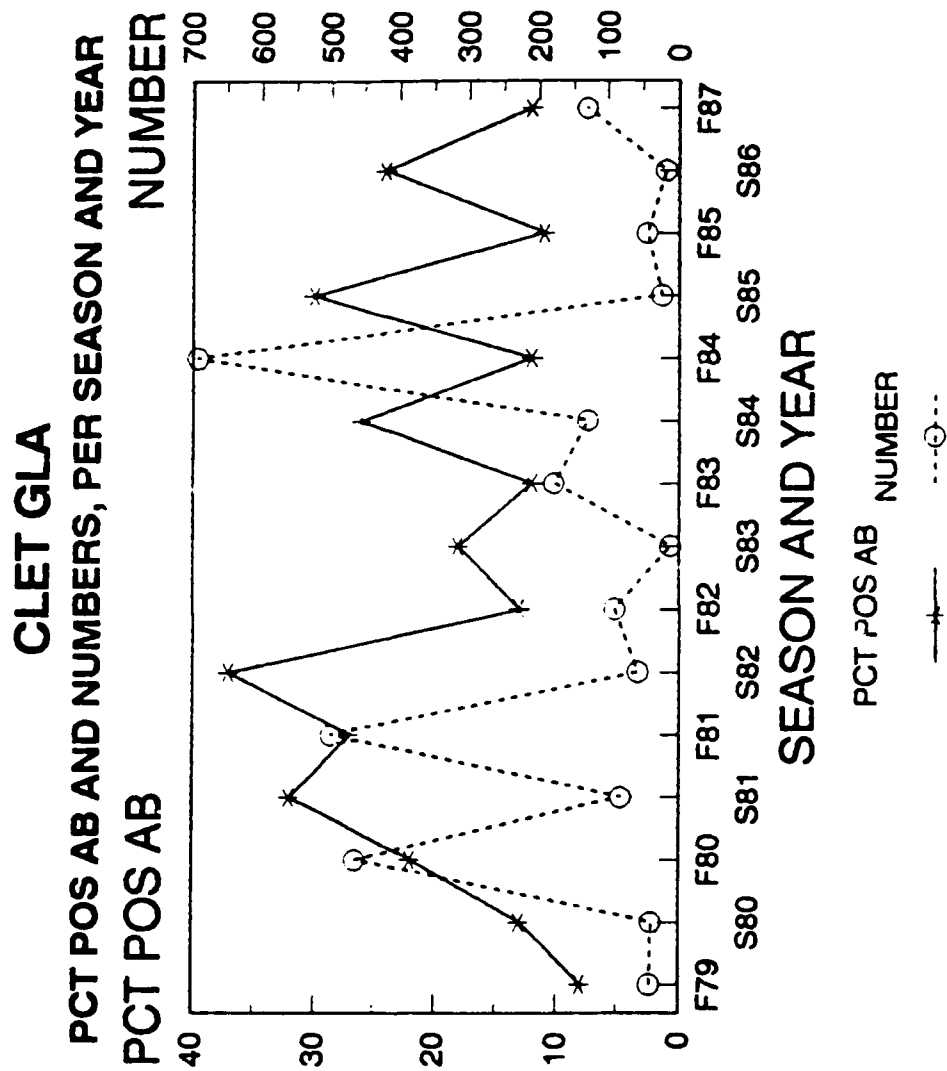


FIGURE 27

# CLET GLA

